JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXII WASHINGTON, D. C., OCTOBER 15, 1921 NO. 3

INFLUENCE OF THE PLANE OF NUTRITION ON THE MAINTENANCE REQUIREMENT OF CATTLE[1]

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In 1914 an investigation was begun at the University of Missouri to study some of the effects of underfeeding. Calves of beef-breeding stock were secured, and they were placed on three planes of nutrition. Group I was fed to grow rapidly, but not to become fat. Group II was placed on a lower nutritive plane and was fed to gain about ½ pound per day. Group III was placed on a still lower nutritive plane and fed to gain about ⅓ pound per day. At the present time three animals remain that were started on the investigation in 1914. Seven others were added in 1917. The older animals, therefore, have been under observation for seven years and the younger animals for four years. Under these circumstances it seemed desirable to make a study of the maintenance requirement of steers at different ages and on different planes of nutrition.[2]

The ideal method of conducting an investigation of this kind would require a respiration calorimeter. Since that was impossible, the alternative was to calculate the energy value of the feed consumed and correct this for the estimated value of the gains or losses in body weight.

The net energy of the feed consumed was calculated in accordance with procedures developed by Armsby.[3] The energy value of the changes in body weight were calculated from the composition of steers that had been analyzed at this Station by the Department of Agricultural Chemistry. So far as possible steers were selected as controls for this purpose that were of similar age, measurements, and weight and that had received similar treatment.

[1] The data for this paper were taken from the thesis of W. D. Salmon, presented at the University of Missouri, as partial fulfillment of the requirements for the degree of Master of Arts. The investigation was initiated by F. B. Mumford, Dean of the College of Agriculture, and by P. F. Trowbridge, at that time Chairman of the Department of Agricultural Chemistry. Since September 1918, E. A. Trowbridge, Chairman of the Department of Animal Husbandry, has had general supervision of the project. This article was prepared by A. G. Hogan, who has been in immediate charge since September, 1920. A large number of workers have contributed to the success of the experiment.

[2] The original data will be reproduced in detail in a subsequent publication.

[3] ARMSBY, H. P., and FRIES, J. A. NET ENERGY VALUES FOR RUMINANTS. Pa. Agr. Exp. Sta. Bul. 142, 20 p. 1916.

METHOD OF THE EXPERIMENT

RATIONS

The concentrate consisted of the following mixture: Corn chop, 60 per cent; wheat bran, 30 per cent; linseed meal, 10 per cent. The roughage fed to the 3 old steers, No. 528, 589, and 585, from the beginning of the experiment until July 20, 1917, was timothy. For the next 10 days a mixture of 5 parts timothy, 3 parts alfalfa, and 2 parts oat straw was fed. Following this the roughage consisted of a mixture of 60 per cent alfalfa amd 40 per cent oat straw. The animals were fed twice daily and had access to water at all times. Salt was accessible at feeding time.

PERIODS

The calculations are made for periods of 180 days, with the exception of the first period for the 3 older steers, which was as follows: No. 528, 130 days; No. 579, 142 days; No. 585, 150 days. The warm months of the year were selected for these periods to avoid a possible disturbing effect of low temperatures in the winter months.

WEIGHTS

The steers were weighed each morning after feeding but before watering. The weight given for the beginning of a period is the average of the 10 preceding days. The weight given at the end is an average of the last 10 days of the period

ENERGY INTAKE

The amount of dry matter consumed was calculated from the weight and composition of the feed consumed. The net energy was computed from this by the use of factors reported by Armsby and Fries.[1] For the concentrates the value 83.82 therms per 100 pounds dry matter was used. This is the factor given for Armsby's grain mixture No. 2,[2] which approximates the grain mixture used in this experiment. For timothy hay the value 48.63 therms per 100 pounds dry matter was used. The factor for the roughage mixture used in the latter part of the experiment was calculated from the Armsby values, for alfalfa, 34.10 therms and for oat straw, 26.03 therms per 100 pounds dry matter. A mixture of 60 parts alfalfa and 40 parts oat straw would have a value of 30.87 therms per 100 pounds dry matter. The calculations of the energy value of the milk are based on factors published by Armsby.[3] There are 29.01 therms per 100 pounds whole milk (4.4 per cent) and 14.31 therms per 100 pounds skim milk (0.2 per cent). From these values factors were computed for the different grades of milk used.

[1] ARMSBY, H. P., and FRIES, J. A. OP. CIT.

[2] Armsby's grain mixture No. 2, 60 per cent corn meal, 30 per cent crushed oats, 10 per cent O. P. linseed meal. Our grain mixture, 60 per cent corn meal, 30 per cent wheat bran, 10 per cent O. P. linseed meal.

[3] ARMSBY, Henry Prentiss. THE NUTRITION OF FARM ANIMALS, p. 719. New York, 1917.

CHANGES IN BODY WEIGHT

In order to obtain data concerning the maintenance requirement of these steers, it is necessary to calculate the energy gained or lost through changes in body weight. Our calculations are based on analyses previously made by the Department of Agricultural Chemistry, University of Missouri.[1] Control animals were selected from those on which analyses were available, on the basis of similar weights and measurements, and when possible of similar ages, daily gains, and daily consumption of dry matter. In some cases suitable control animals were not available, and the composition of steers for those periods was estimated by interpolation, with the exception of the last period for steer No. 528. In this case a value published by Armsby[2] was used. The average energy values of a pound gain as calculated by this method for steers in the three groups are given in Table I. For purposes of comparison the values given by Armsby are shown in the same table.

TABLE I.—*Energy values of a pound gain*

Approximate age (months).	Group I.	Group II.	Group III.	Armsby's values.	
				Age.	Energy.
	Therms.	*Therms.*	*Therms.*	*Months.*	*Therms.*
6	0.95575	0.95575	0.8343	1	1.170
18	1.0918	1.0583	.9445	2 to 3	1.374
36	1.7136	1.1608	1.0548	5 to 6	1.680
54	2.1993	1.4104	1.1013	11 to 12	2.292
66	2.50	1.5352	1.4790	18 to 24	3.000
78	3.00	1.660	1.6490	.....	.....

Armsby's values are consistently higher, as is to be expected. Our animals were thin and contained less than the usual amount of fat in the gain.

In calculating the maintenance requirements per 1,000 pounds live weight, Moulton's[3] formula was used. He has shown that the surface areas of thin cattle are proportional to the $\frac{5}{8}$ power of the live weight. The results of this calculation, on the basis of dry matter consumed, are given in Table II.

The net energy required for maintenance was also calculated by another method,[4] based on the digestible organic matter of the feed.

The following factors are given for the metabolizable energy of digestible organic matter consumed: Roughage, 1.588 therms per pound; grains and similar feeds with less than 5 per cent digestible fat, 1.769 therms per pound. In the same publication the "Average energy expenditure by cattle per 100 pounds of dry matter eaten" is given.

[1] These have not yet been published.

[2] ARMSBY, Henry Prentiss. OP. CIT.

[3] MOULTON, C. R. THE AVAILABILITY OF THE ENERGY OF FOOD FOR GROWTH. *In* Jour. Biol. Chem., v. 31, no. 2, p. 390. 1917.

[4] ARMSBY, H. P., and FRIES, J. A. OP. CIT.

TABLE II.—*Average daily maintenance requirement as calculated from dry matter consumed*

Steer No.	Number of periods averaged.	Therms of net energy per 1,000 pounds, based on ¾ power of live weight.		
		Group I.	Group II.	Group III.
528	6	5.870	.....	.....
577	3	5.280	.....	.....
571	3	5.730	.....	.....
579	5	.....	4.920	.....
578	3	.....	3.830	.....
573	3	.....	4.409	.....
585	5	.....	.....	4.221
575	3	.....	.....	4.041
574	3	.....	.....	4.302
572	3	.....	.....	3.250
Average of all animals for all periods		5.523	4.485	3.830

TABLE III.—*Energy expenditure by cattle per 100 pounds dry matter consumed*

Ration.	Energy expenditure.
	Therms.
Roughage:	
Timothy hay	35.47
Alfalfa hay	53.03
Oat straw	46.00
Concentrate:	
Grain mixture No. 2	51.76

The coefficients of digestibility used in these calculations were derived from digestion trials conducted under similar conditions at this Station. These indicated that the digestibility of the ration varied with the relative amounts of hay and grain fed. The factors used are given in Table IV.

TABLE IV.—*Digestion factors for organic matter*

Ratio of grain to hay.	1:1	2:3	1:2	1:3, 4, or 5	1:6 or 7	1:8, 9, or 10	Hay only.
Factor	.6956	.6695	.6434	.6340	.6229	.6030	0.5832

Inasmuch as the thermal value of a pound of organic matter from grain differs from that of a similar weight of organic matter from roughage, the Armsby factors[1] previously quoted in this paper could not be directly applied to the values obtained with the foregoing digestion coefficients. Those factors would not provide for the widely varying proportions of grain and hay. The following method, therefore, was

[1] ARMSBY, H. P., and FRIES, J. A. OP. CIT.

used in computing the energy intake on the basis of digestible organic matter consumed. By use of the factors in Table IV, the weight in pounds of digestible organic matter in the mixed ration was determined for each period. This was multiplied by 1.588, the Armsby factor for metabolizable energy in a pound of digestible organic matter from hay. The thermal value of digestible organic matter from grain is 1.769, however, or 0.181 therms more. Therefore, each pound of digestible organic matter derived from grain was multiplied by 0.181, and the product was added to the result obtained by multiplying the total digestible organic matter by 1.588. This gave the total metabolizable energy in both the hay and grain. The digestibility of the organic matter of the grain was estimated by difference. This ranged closely around 80 per cent. The factors for energy expenditure are given in Table III.

It seemed impracticable to calculate the net energy of the milk consumed on the basis of digestible organic matter, so the calculation was based on the quantity consumed, as previously described. Since the amount was small, however, the method of calculation would have little effect on the final result.

The method used in correcting for changes in body weight has already been described, and the maintenance requirement as calculated on the basis of digestible organic matter consumed is given in Table V.

TABLE V.—*Average daily maintenance requirement, as calculated from digestible organic matter consumed*

Steer No.	Number of periods averaged.	Therms of net energy per 1,000 pounds based on 5/8 power of live weight.		
		Group I.	Group II.	Group III.
528	6	6.261	.....	.....
577	3	5.412	.....	.....
571	3	5.174	.....	.....
579	5	.....	5.260	.....
578	3	.....	4.192	.....
573	3	.....	4.893	.....
585	5	.....	.....	4.725
575	3	.....	.....	4.454
574	3	.....	.....	4.591
572	3	.....	.....	3.649
	Average of all animals for all periods	5.777	4.869	4.408

In determining the maintenance requirement on the basis of digestible organic matter, the calculations were based on digestion coefficients obtained at this Station under similar conditions. This method is probably more accurate than that of calculation on the basis of dry matter consumed, and for the animals concerned it gives a result about 10 per cent higher.

In calculating average results, obtained by both methods, four periods in which there were losses in live weight were omitted. The results for those periods were low, and we were uncertain as to whether the result was approximately correct or whether it was due to an incorrect assumption as to the energy value of the loss in weight. Most of the dry matter of the loss was probably fat, and if so, our calculation of its energy value was too low and so made our calculation of the maintenance requirement too low.

One steer, No. 585, had a navel infection during the first period, accompanied by a very high maintenance requirement. This period also was discarded in calculating averages.

INFLUENCE OF NUTRITIONAL PLANE

There is a close parallel between the intake of net energy and the maintenance requirement of the animal. The record of steer 574 illustrates that tendency. For the first period the average daily intake of net energy was 3.884 therms per 1,000 pounds, based on the 5/8 power of the live weight; and the maintenance requirement was 3.818 therms. For the second period the energy intake was increased to 5.783 therms, and the maintenance requirement increased to 5.119 therms. In the third period the energy intake was 5.253 therms, and the maintenance requirement was 4.836 therms.

TABLE VI.—*Daily maintenance requirements of cattle—Net energy*

RESPIRATION EXPERIMENTS

Number of experiments.	Investigator.	Condition of animal.	Therms per 1,000 pounds live weight.		
			Maximum.	Minimum.	Average.
22	Armsby and Fries [1]	Medium	7.430	4.723	5.995
7	Kellner [1]	do	6.780	4.921	5.742
	do	Fat	8.871	7.319	7.946

LIVE-WEIGHT EXPERIMENTS

10	Armsby [1]	Thin	7.044	6.136	6.505
3	do	do	6.039	4.713	5.423
6	Haecker [1]	Medium	5.676	4.662	5.021
3	Evvard [1]	do	7.850	6.450	7.180
7	Eckles [1]	do	7.079	5.841	6.173
1	Shirky [2]	do [3]			7.732
2	do	Thin [4]	5.0959	4.953	5.0245
3	Our results	Group I	7.380	4.915	5.777
3	do	Group II	5.724	3.809	4.869
4	do	Group III	5.217	3.276	4.408

[1] ARMSBY, Henry Prentiss. OP. CIT., p. 291.
[2] SHIRKEY, S. B. EXTENT TO WHICH GROWTH RETARDED DURING THE EARLY LIFE OF THE BEEF ANIMAL CAN BE LATER REGAINED. Univ. of Mo. thesis, 1919. (Unpublished.)
[3] Corresponds to group I of this experiment.
[4] Corresponds to group II of this experiment.

In comparing the maintenance requirements of the three groups it should be kept in mind that group I does not represent a high plane of nutrition. The aim was to secure maximum growth with no considerable fattening. Their maintenance requirements as computed in this paper correspond closely to the average of 22 respiration experiments by Armsby and Fries[1] and of 7 by Kellner,[1] on cattle in medium condition. A comparison of our results, and of those obtained by other investigations, is given in Table VI.

INFLUENCE OF AGE

The ages represented in this experiment vary from 30 days for some of the calves at the beginning of the first period to more than 6 years at the close of the seventh period. Apparently there was no relation between the age and the maintenance requirement of these animals. Some of the steers showed a gradual decrease in the maintenance cost from the beginning to the end of the experiment. In such cases it was found that the energy intake per 1,000 pounds had also decreased. On the other hand, steers with an increasing energy intake showed an increased maintenance requirement. Maintenance trials on young animals usually give higher results than have been obtained with mature animals, but if age does influence the maintenance requirement the effect is too slight to be shown in a live-weight experiment of this kind.

SUMMARY AND DISCUSSION

There is a close relation between the amount of net energy consumed and the maintenance requirement. Periods of high energy intake were apparently periods of high maintenance cost, while periods of low energy intake were accompanied by a lowered maintenance requirement.

The averages of the periods discussed show the following daily maintenance requirements per 1,000 pounds live weight, calculated on the basis of digestible organic matter, and in terms of net energy: Group I, 5.777 therms; group II, 4.869 therms; and group III, 4.408 therms. If the maintenance requirement of group I is 100 per cent, that of group II is 84.4 per cent, and that of group III is 76.3 per cent.

The calculations on the basis of dry matter consumed indicate even greater differences. The maintenance requirements as derived by this method may be compared as follows: Group I, 100 per cent; group II, 81 per cent; group III, 69.3 per cent. The estimated maintenance requirement of group I, as calculated by this method, is 30 per cent greater than the total net energy intake of group III.

There is no apparent relation between the age of the animals and the amount of energy required for maintenance.

[1] ARMSBY, Henry Prentiss. OP. CIT.

TURNIP MOSAIC[1]

By MAX W. GARDNER, *Associate in Botany*, and JAMES B. KENDRICK, *Assistant in Botany, Purdue University Agricultural Experiment Station*

In one corner of a small field of turnips near South Bend, Ind., October 12, 1920, a considerable percentage of the plants were found affected with an unmistakable mosaic disease. The symptoms were typical of mosaic diseases in general. The leaves were stunted, misshapen, and a lighter green with dark green blisters or puffy areas. Many of the leaves were extremely distorted by crinkling and folding (Pl. 20, A). The disease seemed to be confined to one area in the field, to some extent coincident with a heavy infestation of tarnished plant bugs.

Several diseased plants were transplanted to pots in the greenhouse, where they continued to form new leaves during the winter. The mosaic symptoms exhibited by the new foliage formed under greenhouse conditions were not quite so extreme as had been noted in the field. One of these plants, with mottled and spindling leaves, is shown in Plate 20, B, as it appeared in December.

Inoculation of a number of potted turnip and radish seedlings was made by breaking off a leaf and rubbing the wound with crushed leaf tissue from one of the mosaic plants. Out of 21 turnip seedlings inoculated early in January, 13 developed characteristic mosaic symptoms. The first symptoms were noted 26 days after inoculation. The turnips inoculated showed some varietal difference from the plants collected in the field in that the leaves were much less distinctly pinnatifid. Out of 46 radish seedlings, including both white and red varieties, similarly inoculated, none developed mosaic symptoms.

A later series of inoculations was made January 26 by wounding the plants with a needle and rubbing the wounded areas with a piece of cotton soaked in the juice from mosaic leaves ground up in a mortar. Ten out of 14 turnip plants thus inoculated developed the mosaic disease. The first symptoms were noted 16 days after inoculation. No mosaic developed among 13 control plants similarly treated except that sterile water was substituted for the mosaic virus. Twenty-two radish plants were also inoculated, and none of these developed the disease. Subsequent reinoculation of turnip plants from one of these radish plants produced no mosaic. The mosaic disease of turnips is therefore readily transmissible to turnips but not to radishes.

[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station, LaFayette, Ind.

After this article was prepared it was learned that Eugene S. Schultz, of the Bureau of Plant Industry, United States Department of Agriculture, was also working on this disease.

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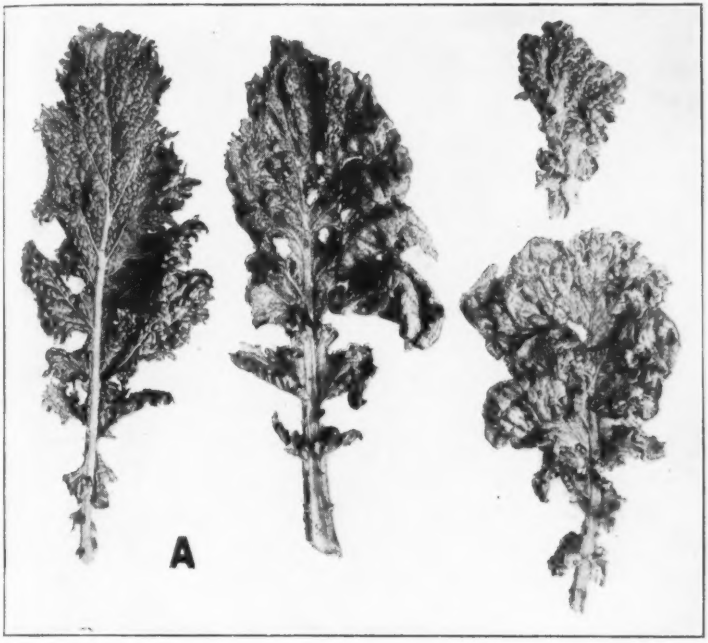
PLATE 20

A.—Leaves from mosaic turnip plants collected October 12, 1920.

B.—Mosaic turnip plant transplanted to a pot in the greenhouse. Photographed December 20, 1920.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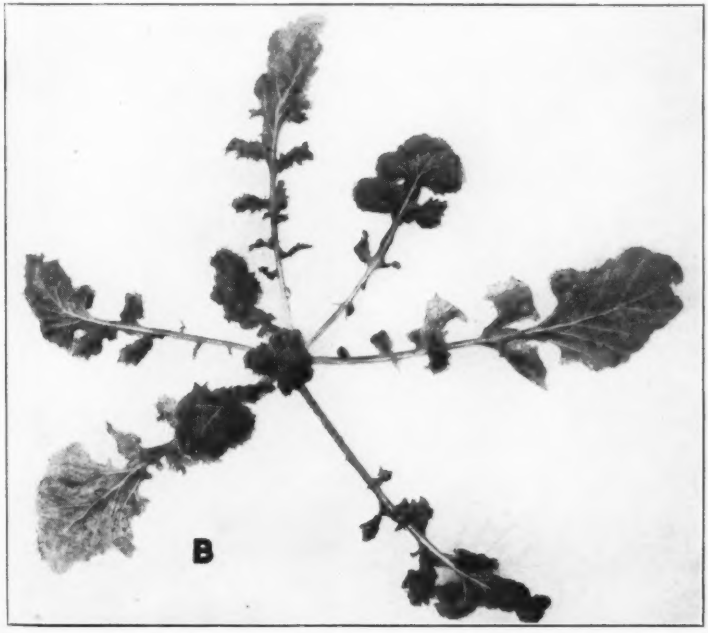
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HYDROCYANIC ACID IN SUDAN GRASS[1]

By C. O. SWANSON [2]

Professor of Agricultural Chemistry, Kansas State Agricultural College

In a previous paper [3] it was shown that hydrocyanic acid (HCN) is obtained from green Sudan grass by macerating, digesting in water, and distilling into a dilute solution of sodium or potassium hydroxid. Several experiments reported in that paper made it clear that this acid does not exist free in Sudan grass and is obtained only if the conditions of the determination are favorable to enzym action. It appears to be a common belief that hydrocyanic acid is developed by freezing. This merely bursts the green cells and thus performs the same function as maceration, with the result that the hydrocyanic acid is rapidly lost from frosted grass. It was also shown that while in some cases poisoning had been reported from pasturing Sudan grass, under normal conditions no poisoning took place either before or after the grass was frozen. It was suggested that when frozen the hydrocyanic acid had been liberated and then evaporated as the grass dried.

Because of the importance of the subject it was thought worth while to make further investigations. During the summer of 1920, material was obtained from a 1/20-acre plot of Sudan grass grown by the Department of Agronomy of the Kansas State Agricultural College. The Sudan grass had been planted early in June in rows about 2 feet apart. On June 22, when the experiments were begun, the grass was about 6 inches high. These experiments were continued during the summer and early fall.

METHOD OF DETERMINING HYDROCYANIC ACID

At present there are no satisfactory quantitative methods for estimating hydrocyanic acid obtained from organic material. All are open to some objection. After considering several, the Prussian-blue method was adopted as best suited for the purposes of the present investigation. Because of simplicity in manipulation it is possible to run a large number of determinations at the same time. The amount of hydrocyanic acid obtained from the different samples was estimated colorimetrically, using standard solutions containing known amounts of potassium cyanid. One objection to the colorimetric measurements was the difficulty in

[1] Contribution No. 92 from the Department of Chemistry, Agricultural Experiment Station of Kansas State Agricultural College.

[2] Credit is due Mr. Carl M. Conrad for efficient assistance in making the determinations reported in this paper.

[3] SWANSON, C. O. HYDROCYANIC ACID IN SUDAN GRASS AND ITS EFFECT ON CATTLE. *In* Jour. Amer. Soc. Agron., v. 13, no. 1, p. 33-36. 1921.

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obtaining a uniform blue color. Very often the precipitate was decidedly green. It was found that by warming and letting the precipitate stand for some time in loosely stoppered bottles a uniform blue color could be obtained. The use of nitric or sulphuric acid instead of hydrochloric acid or the addition of potassium fluorid, all of which have been suggested by other workers, did not seem to eliminate, entirely, the green color. While the defects of the Prussian-blue method are fully realized, it compares favorably with other methods.[1] In no sense are the values reported in this paper to be regarded with the same degree of accuracy as a protein or even a crude-fiber determination. For this reason no conclusions should be drawn from the results unless the figures presented are uniformly consistent or the differences large.

The calculations in this paper are based upon approximately 200 gm. of green material. When the grass was wilted or dry the weight of sample used was proportionately lessened. It is impracticable to secure green samples of uniform weights of dry matter, particularly if they are gathered during different hours of the day and throughout several weeks and months. Then, as will be shown in what follows, the hydrocyanic acid is localized in the plant, being present in the largest amounts in those portions of the plant possessing the greatest vegetative activity. For this reason leaves were separated from the stems whenever these were present. The amount of hydrocyanic acid obtained is small in proportion to the total weight of samples used. It was seldom more than 0.015 per cent.

EFFECTS OF MACERATION

The first sample was collected June 22, when the grass was about 6 inches high. This was cut into pieces about ¼ inch long and digested for three hours in water at room temperature. Less than 1 mgm. hydrocyanic acid was obtained. Another sample, taken the next day, was cut and thoroughly macerated by pounding in an iron mortar and was then digested in water. This sample gave 27 mgm. hydrocyanic acid. On June 28 a sample was secured and divided into two equal portions. One portion was cut and macerated as described above, and the other was cut and macerated with coarse, sharp sand. Both were digested in water for the same length of time. The portion macerated with sand gave 26 mgm. hydrocyanic acid, and the other gave 36 mgm. It appeared from this that maceration with sand was not necessary and might result in a loss. Subsequent experiments showed that as soon as the grass is macerated the hydrocyanic acid is liberated and for this reason may be lost. On August 18 a sample was divided into four portions and, after the preliminary treatment mentioned, was digested overnight, with the result given in Table I.

[1] VIEHOVER, Arno, and JOHNS, Carl O. ON THE DETERMINATION OF SMALL QUANTITIES OF HYDROCYANIC ACID. *In* Jour. Amer. Chem. Soc., v. 37, no. 3, p. 601-607. 1915.

TABLE I.—*Effect of maceration on liberation of hydrocyanic acid*

Sample No.	Treatment.	HCN.
		Mgm.
1	No cutting or maceration	0
2	Cut in feed cutter (¼ to ⅛ inch)	10
3	Cut and macerated slightly	10
4	Cut and macerated thoroughly	11

From this it appeared that if the time of digestion is sufficiently long the amount of maceration is less important, provided, however, that the plant tissue is cut fairly fine. The smaller amount obtained from the grass on August 18, as compared with that obtained in June, is in accord with a general observation made during the summer, that as the season advanced smaller amounts were obtained from the 200-gm. portions.

TIME REQUIRED FOR DIGESTION

The time required for digestion in order to obtain the maximum amount of hydrocyanic acid was determened. A sample collected on June 28 was divided into three portions and similarly treated, except for the time allowed for digestion. The results obtained are given in Table II.

TABLE II.—*Effect of time of digestion on liberation of hydrocyanic acid*

Sample No.	Treatment.	HCN.
		Mgm.
1	Digested 3 hours	18
2	Digested 6 hours	32
3	Digested 24 hours	32

This experiment seemed to show that digesting 3 hours was not long enough, while 6 hours was as effective as 24. On August 28 a similar experiment gave the results shown in Table III, the results in each case being an average of duplicate samples.

TABLE III.—*Effect of time of digestion on liberation of hydrocyanic acid*

Sample No.	Treatment.	HCN.
		Mgm.
1	Digested two days	10
2	Digested three days	10
3	Digested four days	10
4	Digested seven days	0

The portion digested seven days developed a very bad odor. Because of these results, the usual procedure with experiments reported in this paper was to macerate the sample and then digest at room temperature overnight.

LOCALIZATION OF HYDROCYANIC ACID IN THE PLANT

At three different times the grass was divided into leaves and stems. From 200-gm. portions the number of milligrams of hydrocyanic acid shown in Table IV were obtained.

TABLE IV.—*Hydrocyanic acid in leaves and stems of Sudan grass*

Date collected.	Leaves.	Stems.
	Mgm.	*Mgm.*
June 30	67	5
July 7	33	Trace.
July 21	66	0

The immaturity of the sample collected June 30 accounts for the moderate amount obtained from the stems. At a later date two tests were made on immature heads. No hydrocyanic acid was found. In the following tests reported in this paper leaves only were used unless otherwise stated.

INFLUENCE OF STAGE OF GROWTH

Since grass was cut almost every week throughout the summer there were afforded several opportunities to test the comparative amounts present in various stages of growth and development. The shorter grass was obtained from plants which had been cut once or several times.

TABLE V.—*Hydrocyanic acid in Sudan grass at different dates and stages of growth*

Date collected.	Portion used.	Average height.	HCN.
			Mgm.
July 22	Whole plant	6 inches	27
	....do	8 inches	19
	....do	12 inches	7
	Leaves	18 inches	24
	....do	24 inches	20
	....do	30 inches	9
26	Whole plant	4 inches	40
	do	6 inches	32
	do	12 inches	10
	Leaves	Beginning to head	12
	do	Partly headed	17
	do	Fully headed	20
	do	Partly dead	6
31	Whole plant	4 inches	11
	Leaves	16 inches	18
	do	Headed	19
Aug. 12	Whole plant	4 inches	40
	do	10 inches	12
	Leaves	15 inches	16
	do	24 inches	10
27	Whole plant	5 inches	10
	Leaves	20 inches	11
30	Whole plant	2 inches	5
	do	12 inches	5
	Leaves	Ready to head	9
	do	Blooming	10

The results show that more hydrocyanic acid is found in the whole plant in the earlier stages of growth and less as the season advances. The difference is perhaps due to the large proportion of stems in the latter part of the season, since if leaves only are compared there is very little difference except where they are from mature plants. This indicates that most of the hydrocyanic acid is obtained from those parts of the plants where the vegetative activity is most pronounced. This agrees with the results obtained by Menaul and Dowell [1] at the Oklahoma Agricultural Experiment Station. These observations support the theory that hydrocyanic acid is an intermediate product between the nitrates and the amino acids.[2]

DISAPPEARANCE FROM MACERATED MATERIAL

As soon as the grass is macerated the hydrocyanic acid begins to pass off. This was demonstrated several times by suspending small pieces of sodium-picrate paper above some macerated grass in stoppered flasks. The paper very soon assumed a brown color. The quantitative determinations given in Table VI were made on samples macerated July 10 and treated as indicated.

TABLE VI.—*Disappearance of hydrocyanic acid in macerated grass*

Sample No.	Treatment.	HCN.
		Mgm.
1	Digested in water two days	25
2	Placed without added water in covered mason jar for two days then small amount of water added and distilled	12
3	Placed in flask two days so that the hydrocyanic acid could escape only into the receiving flask, after which water was added and distilled	26
4	Left in open jar for two days, digested and distilled	Trace.
5	Repeat of 3 but kept in flask overnight only	26

DISAPPEARANCE FROM GRASS AFTER CUTTING

In a previous paper [3] it was stated that tests made on partially wilted grass may be worthless. In the experiments made at that time, the amount of sulphuric acid added was not carefully enough controlled. It will be shown in the following paragraphs that if acid is added beyond certain limits no hydrocyanic acid will be obtained from either green or partially wilted grass. In each of the determinations given in Table VII the grass was macerated after the treatment stated and then digested in water overnight.

[1] MENAUL, Paul, and DOWELL, C. T. CYANOGENESIS IN SUDAN GRASS: A MODIFICATION OF THE FRANCIS-CONNELL METHOD OF DETERMINING HYDROCYANIC ACID. *In* Jour. Agr. Research, v. 18, no. 8, p. 447-450. 1920.

[2] RAVENNA, C., and ZAMORANI, M. NUOVE RICERCHE SULLA FUNZIONE FISOLOGICA DELL, ACIDO CIANIDRICO NEL SORGHUM VULGARE. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., v. 18, sem 2, no. 8, p. 283-287. 1909. Abstract *in* Chem. Abs., v. 5, no. 6, p. 1123. 1911.

[3] SWANSON, C. O. HYDROCYANIC ACID IN SUDAN GRASS AND ITS EFFECT ON CATTLE. *In* Jour. Amer. Soc. Agron., v. 13, no. 1, p. 33-36. 1921.

TABLE VII.—*Disappearance of hydrocyanic acid from Sudan grass after cutting*

Date collected.	Sample No.	Treatment.	HCN.
			Mgm.
June 29	1	Wilted in shade	28
	2	Green, control sample	36
July 7	3	Wilted in sun for three hours	12
	4	Dried in sun from morning till evening, outdoors overnight	15
	5	Dried in shade for same length of time as 2	24
	6	Dried outdoors two days and nights	15
	7	Dried in the shade two days and nights	7
	8	Dried in shade three days and nights	20
Aug. 12	9	Dried in the shade five days	32
Sept. 1	10	Dried in the shade two days	6

While these results are not uniform, they do show conclusively that hydrocyanic acid can be obtained from wilted grass. Because of this result an attempt was made to determine more accurately the amount of hydrocyanic acid that may be obtained from wilted and dried grass.

EFFECTS OF KEEPING GREEN GRASS MOIST AFTER IT IS CUT

A large sample of grass collected June 29 was placed stems down in a large bottle so that about one-fourth was immersed in water. At the end of different periods of time 200-gm. portions of the leaves were macerated and digested in water overnight. The amount of hydrocyanic acid obtained is given in Table VIII.

TABLE VIII.—*Effect of keeping grass moist after cutting*

Sample No.	Length of treatment.	HCN.
		Mgm.
1	6 hours	32
2	22 hours	20
3	30 hours	8
4	48 hours	2

The results indicate that hydrocyanic acid slowly disappears from the grass after it is cut, but also that the grass may be kept for a while in the green condition without much loss of the hydrocyanic acid. Control samples taken at this time gave 30 mgm. hydrocyanic acid.

When the grass was wholly covered with water or when the air was excluded the results were different. In each case in the experiment reported in Table IX, unless otherwise stated, the grass was macerated and digested at the end of the treatment given.

This shows that the presence or absence of air has an intimate relation to the evolution of hydrocyanic acid. Experiments were performed in which the grass was kept in an atmosphere of carbon dioxid and also of

hydrogen. No hydrocyanic acid was obtained from the grass kept in an atmosphere of hydrogen, whereas from that kept in carbon dioxid considerable amounts were obtained. Some macerated grass was also placed in a desiccator from which the air was exhausted continuously. This did not seem to affect the amount of hydrocyanic acid obtained, but the experiment was not satisfactory. The effect of keeping the grass in different atmospheres needs further study.

TABLE IX.—*Effect of different treatments after cutting on hydrocyanic acid content*

Date collected.	Treatment.	HCN.
		Mgm.
July 8	Placed uncut in bottles and covered with water two days	½
	Distillate from this water	1
	Placed uncut in sealed mason jar with small amount of water two days	1
	Placed uncut in sealed mason jar with small amount of chloroform two days	12
	Placed uncut in sealed mason jar for 2 days, no water	8
	Placed uncut in bottle 2 days, covered with water	2
July 10	Obtained by distilling water from this	2
	Macerated and digested 2 days in water	25
	Macerated and placed in bottle 2 days then water added and distilled	12
	Macerated and placed in open pan 2 days	Trace.

EFFECT OF HOT WATER

To determine this relation, enough grass was cut to make twenty-four 200-gm. portions of leaves. After the preliminary treatments as indicated in Table X, one set of 12 samples was digested in cold water and another set of 12 samples in hot water.

TABLE X.—*Effect of adding hot water on amount of hydrocyanic acid obtained*

Time of drying in shade. (hours).	Treatment before digestion.	Time of digestion.	HCN obtained after adding—	
			Water at room temperature.	Boiling water
			Mgm.	*Mgm.*
0	Uncut	5 hours	0	0
	Cut in feed cutter	do	0	0
	Macerated	do	10	Trace.
7	Uncut	24 hours	7	0
	Cut in feed cutter	do	14	0
	Macerated	do	34	0
28	Uncut	Overnight	8	0
	Cut in feed cutter	do	12	1
	Macerated	do	19	1
	Uncut	30 hours	4	Trace.
	Cut in feed cutter	do	10	2
	Macerated	do	19	2

This shows that it is possible to obtain some hydrocyanic acid from the uncut green grass if the time of digestion is sufficiently long. In every case more was obtained when the material was cut in the feed cutter and still more when it was macerated. Hot water placed on the green material entirely prevented liberation. The small amount obtained from the partially wilted grass when the hot water was added was probably in a free condition at the time of adding the hot water. Almost as much hydrocyanic acid was obtained from the grass that was wilted seven hours as from the fresh grass if digested in water at room temperature sufficiently long.

This experiment as well as several others show that under some circumstances it is possible to obtain hydrocyanic acid from wilted or dried grass both with and without digestion in either hot or cold water. To investigate this further the following experiment was planned and executed. Five sets of 12 samples were secured and treated as follows: (1) Dried in the sun; (2) dried in the shade; (3) exposed in the sun, but kept moist by frequent sprinkling with water; (4) exposed in the shade but kept moist by sprinkling with water; (5) frozen in an ice machine and then exposed in open pans in the shade. The duration of these treatments was for 4, 8, 24, 31, and 48 hours, respectively. Six of the samples from each set were macerated after the period of the preliminary treatment, and hot water was poured on and distilled at once. The other six were digested in cold water overnight and then distilled. The results are shown in Table XI.

TABLE XI.—*Rate of disappearance of hydrocyanic acid from Sudan grass after it is cut and variously handled*[a]

Time of treatment.	Hours of preliminary treatment.	Dried in sun, treated with—		Exposed in sun but kept wet and treated with—		Dried in shade and treated with—		Exposed in shade but kept wet and treated with—		Frozen before exposed in shade and treated with—	
		Hot water.	Cold water.	Hot water	Cold water	Hot water	Cold water.	Hot water.	Cold water	Hot water	Cold water
9 a. m first day.....	0	0	16	.....	.....	0	16	0	14	2	8
9 a. m. first day to 1 p. m. first day.....	4	6	12	Trace.	4	4	14	.....	.....	Trace.	8
9 a. m. first day to 5 p. m. first day.....	8	5	10	8	8	1	10	.....	.....	0	6
9 a. m. first day to 9 a. m. second day.....	24	6	10	1	4	2	6	9	5	1	Trace.
9 a. m. first day to 5 p. m. second day....	31	½	3	Trace.	6	6	5	8	8	0	Trace.
9 a. m. first day to 9 a. m. third day.....	48	Trace.	Trace.	0	Trace.	5	7	4	10	0	10

[a] The figures indicate milligrams of hydrocyanic acid from 200 gm. of grass and are averages of several determinations.

The results show that no hydrocyanic acid is obtained from green material when treated with hot water very soon after cutting and macerating, but that when the grass is wilted as much as four hours in the sun, considerable hydrocyanic acid is obtained by treating with hot water immediately after maceration. The amount of hydrocyanic acid

obtained was not greater when the grass was wilted for a longer time. Less hydrocyanic acid is obtained from grass that is kept moist while in the sun than from grass that is allowed to dry rapidly. According to Ravenna and Zamorani [1] the nitrogen passes through the following stages in the plant: Nitrate → hydrocyanic acid → amino substance → protein substance. According to this theory the cells which continue to be active use the hydrocyanic acid for the building of protein substance, and as more nitrates from the soil are not supplied for manufacture of more hydrocyanic acid, the potential amount present when the plant is cut is soon exhausted.

When the grass was dried slowly in the shade the hydrocyanic acid disappeared more slowly than when it was dried in the sun, and the amount obtained from the hot-water treatment became approximately equal to that obtained from the longer digestion in cold water. This seems to mean that when the plant wilts the hydrocyanic acid is split off from glucocids and held in such loose combination that it can be set free by hot water and that practically all the hydrocyanic acid is in such combination, since additional amounts can not be obtained by further digestion. Splitting off begins as soon as the plant is cut. Determinations made on grass kept moist in the shade appear to show that after 24 hours all the hydrocyanic acid not otherwise used by the cells is in such a condition that it is soluble in water.

In the test in which the grass was frosted the hydrocyanic acid disappeared very rapidly, though the results were not very consistent.

EFFECT OF ACIDS

On June 24 a sample of grass was placed in a flask after maceration, covered with water, and sulphuric acid was added to acid reaction. After it was digested and distilled as usual only a trace of hydrocyanic acid was obtained. From a sample of like material and similarly treated, except that no acid was added, 27 mgm. were obtained. On June 29 this experiment was repeated with the result that 8 mgm. were obtained when acid was used and 26 mgm. when it was not used. On June 30, 1 and 28 mgm. were obtained by these respective treatments. These experiments clearly indicate that the presence of acid has a very importance influence on the amount of hydrocyanic acid that may be obtained. To test the effect of the amount of acid used, four samples were prepared on July 14 and digested overnight in the following: (1) water; (2) *N/o.1* sulphuric acid (H_2SO_4); (3) *N/o.2* sulphuric acid; (4) *N/1* sulphuric acid. No hydrocyanic acid was obtained from any of the treatments with sulphuric acid, whereas the water digestion gave 30 mgm. On August 4 this experiment was repeated, using a weaker acid solution. Digestion in water gave 10

[1] RAVENNA, C., and ZAMORANI, M. NUOVE RICERCHE SULLA FUNZIONE FISIOLOGICA DELL' ACIDO CIANIDRICO NEL SORGHUM VULGARE. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., v 18, sem. 2, no. 8, p. 283–287. 1909. Abstract *in* Chem. Abs. v. 5, no. 6, p. 1123. 1911.

mgm. of hydrocyanic acid; *N/0.01* sulphuric acid, 11 mgm.; and in *N/ 0.02* sulphuric acid, 4 mgm. On August 2 three samples were prepared and digested in *N/1* sulphuric acid; in *N/0.2* sulphuric acid; and in *N/0.05* sulphuric acid. Just before distillation, sodium hydroxid was added to almost neutral reaction. From the *N/0.05* sulphuric acid 18 mgm. of hydrocyanic acid were obtained; a trace was obtained from the *N/0.2*, and none from normal. The weakest of the acid solutions gave no more than water alone. The smaller amounts obtained from the water treatments at the later date is in accord with the general observation that as the season advanced less hydrocyanic acid was present. It was planned to determine the exact hydrogen-ion concentration at which the hydrocyanic acid is most easily split off, but time did not permit. It is hoped that this may be determined in the future.

It was shown in connection with the hot-water treatment that when grass dries the hydrocyanic acid is changed into a free condition, so that simply adding hot water and distilling will drive off the hydrocyanic acid. To see if more would be driven off if acid was also present the following experiment was performed. Six samples of leaves were placed in the open in clear weather from 9 a. m. till 9 a. m. the next day. Then they were macerated and digested in water and in different concentrations of sulphuric acid. The results are given in Table XII.

TABLE XII.—*Effect of acid solutions in formation of hydrocyanic acid*

H_2SO_4 added.	HCN obtained.	H_2SO_4 added.	HCN obtained.
	Mgm.		*Mgm.*
N/1	0	*N/0.02*	Trace.
N/0.2	0	*N/0.01*	10
N/0.05	0	Water	18

Thus, it appears that sulphuric acid is unfavorable to the liberation of the hydrocyanic acid even in the wilted material.

To determine whether hot sulphuric acid would liberate the hydrocyanic acid, hot water and sulphuric acid of varying normalities were added to green material immediately after maceration on July 16. The results are shown in Table XIII.

TABLE XIII.—*Effect of hot sulphuric acid on formation of hydrocyanic acid*

H_2SO_4 added.	HCN obtained.	H_2SO_4 added.	HCN obtained.
	Mgm.		*Mgm.*
N/1	Trace.	*N/0.05*	0
N/0.5	0	*N/0.02*	Trace.
N/0.2	0	*N/0.01*	Trace.
N/0.1	Trace.	Hot water	5

This shows that the use of hot acid is similar to that of hot water and that acid has no power to split off the hydrocyanic acid, at least in the concentration used. The traces obtained in some cases were no doubt due to liberation of hydrocyanic acid during maceration. It would, appear, however, that hot water was less destructive than hot acid. Like experiments with hydrochloric acid were performed with similar results.

On July 21 eight samples were prepared and digested at room temperature in phosporic acid (Table XIV).

TABLE XIV.—*Effect of phosphoric acid on liberation of hydrocyanic acid*

H_3PO_4 added.	HCN obtained.	H_3PO_4 added.	HCN obtained.
	Mgm.		*Mgm.*
N/1	0	*N*/0.05	0
N/0.5	0	*N*/0.02	8
N/0.2	0	*N*/0.01	8
N/0.1	0	Water	8

The results indicate that the inhibiting power of phosphoric acid (H_3PO_4) was somewhat less than that of hydrochloric (HCl) or sulphuric acid. This would be expected since the degree of ionization of phosphoric acid is less than that of hydrochloric or sulphuric acid. Experiments with tartaric acid gave similar results.

EFFECT OF DIGESTING IN ALKALINE SOLUTION

On July 20, 16 samples were prepared and digested in sodium-hydroxid (NaOH) and sodium-carbonate (Na_2CO_3) solutions, respectively (Table XV).

TABLE XV.—*Effect of alkaline solution on formation of hydrocyanic acid*

NaOH added.	HCN obtained.	Na_2CO_3 added.	HCN obtained.
	Mgm.		*Mgm.*
N/1	0	*N*/1	0
N/0.5	0	*N*/0.5	0
N/0.2	0	*N*/0.2	0
N/0.1	0	*N*/0.1	0
N/0.05	0	*N*/0.05	Trace.
N/0.02	11	*N*/0.02	17
N/0.01	18	*N*/0.01	17
Water	19	Water	20

The results show the same general effect as that secured with acid solutions.

EFFECT OF ACID OR ALKALI ON HYDROCYANIC ACID AFTER IT IS LIBERATED

An experiment was performed to show what effect acid or alkaline solutions have on the hydrocyanic acid after it is liberated. The green, macerated material was digested overnight in measured amounts of water. Enough standardized acid or alkali was then added to give the normality desired, and distilled. The results are given in Table XVI.

TABLE XVI.—*Effect of acid and alkali on hydrocyanic acid after it is liberated*

Solution added.	HCN obtained after treatment with solutions of—				
	N/1.	*N/0.2.*	*N/0.1.*	*N/0.05.*	Water.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
H_2SO_4	10	17	.....	14	20
Hcl	9	13	16	18	32
H_3PO_4	16	8	20	18	32
NaOH	11	11	2	0	20

No hydrocyanic acid passed over in the first distillate from the sodium-hydroxid solution. The mixture was acidified with sulphuric acid and then distilled with the results given in Table XVI. While the results obtained in this experiment are not very uniform, they do show that hydrocyanic acid can be obtained from acid and alkaline solutions if the hydrocyanic acid is in a free condition before the acids are added. The experiment also appears to show that the addition of acid or alkali resulted in diminishing the amount of hydrocyanic acid obtained. The experiment was also tried by digesting the grass in sulphuric acid and sodium hydroxid of the normalities *N/1*, *N/0.2*, *N/0.1*, and *N/0.05* and then neutralizing before distilling. In no case was any hydrocyanic acid obtained.

INFLUENCE OF WEATHER

From a sample taken June 23, when there had been no rain for three weeks, 27 mgm. of hydrocyanic acid were obtained. On June 30, after a heavy rain and a week of good growing weather, during which there was plenty of moisture, 30 mgm. were obtained. On July 24, when there had been a period of dry weather, the amount obtained was 7 mgm. The next day, following a rain during the night, the amount was 16 mgm. Several experiments indicated that the largest quantity was obtained when the plant was in the most vigorous growing condition. This is contrary to a common belief that stunting has some effect in increasing hydrocyanic acid. On the contrary, the potential amount may be lessened. Determinations were made on samples collected at sundown and also before sunrise. The data obtained were not conclusive in determining the effect of light on the potential amount of hydrocyanic acid present.

HYDROCYANIC ACID IN SUDAN HAY

Two samples were taken from the outside of a stack of Sudan hay and two from the inside. No hydrocyanic acid was found.

AMOUNT OF HYDROCYANIC ACID IN OTHER SORGHUMS

On July 23 a sample of kafir was taken and separated into leaves and stems. From the leaves were obtained 16 mgm., and from the stems 10 mgm. of hydrocyanic acid. The kafir stems were very little developed. Sudan grass, tested the same day, gave 8 mgm. of the acid from the same weight of material. On July 26, just after a heavy rain, following a period of dry weather, a sample of kafir gave 72 mgm. and a sample of sorgo (cane) 42 mgm. of hydrocyanic acid. Sudan grass 6 inches high, tested on that date, gave 32 mgm. of hydrocyanic acid.

On August 7 a sample of second-growth sorgo (cane) was received from LaHarpe, Kans. About one-fifth was quite dry, two-fifths were wilted and yellow, and two-fifths were green. The sample was somewhat moldy. One portion digested in the usual manner gave 13 mgm. hydrocyanic acid. Another portion distilled at once from hot water gave 24 mgm., showing that the hydrocyanic acid was in free condition. Another sample of sorgo was sent in from Seneca, Kans. This was reported to have killed six cows. From the portion distilled from hot water 20 mgm. were obtained and from the portion digested in the usual way 36 mgm.

On September 2 a quantity of Red Amber kafir was collected, and six portions were prepared and treated with the results given in Table XVII.

TABLE XVII.—*Hydrocyanic acid in Red Amber kafir*

Sample No.	Treatment.	HCN.
		Mgm.
1	Left in flask 15 minutes after maceration, after which hot water was added and distilled	32
2	Digested overnight in water	119
3	Digested overnight in *N*/0.5 H_2SO_4	None.
4	Digested overnight in *N*/0.1 H_2SO_4	None.
5	Digested overnight in *N*/0.01 H_2SO_4	40
6	Digested overnight in *N*/0.01 NaOH	36

Part of this experiment was repeated by putting macerated sorgo into boiling water at once. This gave 8 mgm. hydrocyanic acid, while that digested overnight gave 96 mgm. Another portion was divided into five portions. After maceration they were all digested in water overnight. Then to these portions standardized sulphuric acid was added so as to make the normalities indicated. The results are given in Table XVIII.

These determinations show without a doubt that sorgo and kafir contain much larger amounts of hydrocyanic acid than does Sudan grass, and also that the conditions for obtaining it are very similar.

TABLE XVIII.—*Effect of different treatments on liberation of hydrocyanic acid in sorgo*

Sample No.	Treatment.	HCN.
		Mgm.
1	Digested in water and distilled	72
2	Digested in water and distilled	80
3	Digested in water and distilled from *N*/0.5 H_2SO_4	64
4	Digested in water and distilled from *N*/0.1 H_2SO_4	72
5	Digested in water and distilled from *N*/0.02 H_2SO_4	89

EFFECT OF HYDROCYANIC ACID FROM GREEN SORGO ON A HORSE

Ten-pound portions of green sorgo, testing the amount of hydrocyanic acid given in Table XVIII, were fed to a horse. No effect on respiration, pulse, or temperature could be observed by Dr. H. F. Lienhardt, of the Veterinary Division, who made the observations. Data presented in this paper show that such a degree of acidity as is found in the stomach of a horse would prevent liberation of hydrocyanic acid from the green material. Feeding wilted sorgo was not tried.

SUMMARY

(1) In this paper are presented data giving the results of tests made on Sudan grass for hydrocyanic acid during the summer and early fall of 1920.

(2) The maximum amount of hydrocyanic acid was obtained by macerating the material and digesting in water at room temperature for about six hours or overnight.

(3) Practically all the hydrocyanic acid was found in the leaves. In well-developed stems none was found.

(4) More hydrocyanic acid was found in younger plants than in those more mature. This is due mostly to stem development. If leaves only are used the differences are small, except when the plants approach maturity. More was found in the summer than in the fall.

(5) Hydrocyanic acid does not exist as free HCN in the growing plant. It begins to be liberated as soon as the plant is macerated or undergoes wilting.

(6) Liberation of hydrocyanic acid is intimately associated with enzym action. If this enzym action is inhibited by addition of hot water or acids, no hydrocyanic acid will be liberated. Hydrocyanic acid was obtained from wilted grass when hot water was added, because during the wilting process hydrocyanic acid was set free.

(7) Hydrocyanic acid can not be set free from the green material by acids.

(8) The action of strong alkali is similar to that of acids.

(9) Most hydrocyanic acid is present when the plant is in a vigorous growing condition.

(10) Sudan grass contains less hydrocyanic acid than sorgo or kafir.

NUTRIENT REQUIREMENTS OF GROWING CHICKS: NUTRITIVE DEFICIENCIES OF CORN[1]

By F. E. MUSSEHL, *Professor of Poultry Husbandry*, J. W. CALVIN, *Associate Chemist*, *Nebraska Agricultural Experiment Station*, with the cooperation of D. L. HALBERSLEBEN and R. M. SANDSTEDT

Investigators in the field of nutrition have noted that chickens behave unlike rats and swine when limited to rations of corn or wheat grains and their products. This fact has made necessary the planning and execution of experimental work having for its object a determination of the values and deficiencies of our common feeding stuffs when used for poultry and egg production. The results of a series of experiments carried on at this Station with this objective are reported in this paper.

From the experience of investigators[2] who have worked with other species, mainly rats and swine, it has seemed that systematic inquiry should be made into the (a) ash requirements, (b) protein requirements (quality and quantity), and (c) food accessory requirements. Earlier investigational work with chicks by Osborne and Mendel[3] and Hart, Halpin, and Steenbock[4] indicates that another element, (d) the physical factor, is also of fundamental importance and must be considered in any complete study of the nutritive values of a particular grain or ration.

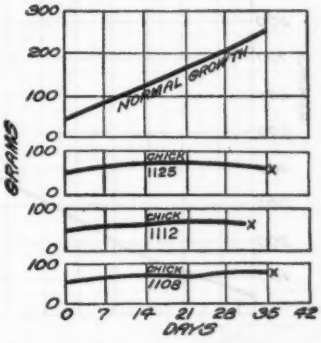


FIG. 1.—Graph showing unsatisfactory results from feeding ration of 100 parts yellow corn and calcium carbonate grit ad libitum to chicks of lot 11. The time at which chicks died is indicated by *X*.

In our work 10-day-old Single-Comb White Leghorn chicks were used, special care being taken to select for vigor, vitality, and uniformity in each lot. Nine chicks per lot were used for the first series of experiments. Chicks were weighed individually every seven days, and the growth curves selected are typical of each lot (fig. 1–11). They show the weight of the chicks at the beginning of the experiment and the change in weight thereafter. Records of the feed

[1] Published with the approval of the Director of the Nebraska Agricultural Experiment Station.

[2] MCCOLLUM, E. V., SIMMONDS, N., and PITZ, W. THE RELATION OF THE UNIDENTIFIED DIETARY FACTORS, THE FAT-SOLUBLE *a*, AND WATER-SOLUBLE *b*, OF THE DIET TO THE GROWTH-PROMOTING PROPERTIES OF MILK. *In* Jour. Biol. Chem., v. 27, no. 1, p. 33-43, 6 charts (1-3, 6 in text). 1916.

[3] OSBORNE, Thomas B., and MENDEL, Lafayette B. THE GROWTH OF CHICKENS IN CONFINEMENT. *In* Jour. Biol. Chem., v. 33, no. 3, p. 433-438, pl. 4-6. 1918.

[4] HART, E. B., HALPIN, J. G., and STEENBOCK, H. USE OF SYNTHETIC DIETS IN THE GROWTH OF BABY CHICKS. A STUDY OF LEG WEAKNESS IN CHICKENS. *In* Jour. Biol. Chem., v. 43, no. 2, p. 421-442, 2 pl. 1920.

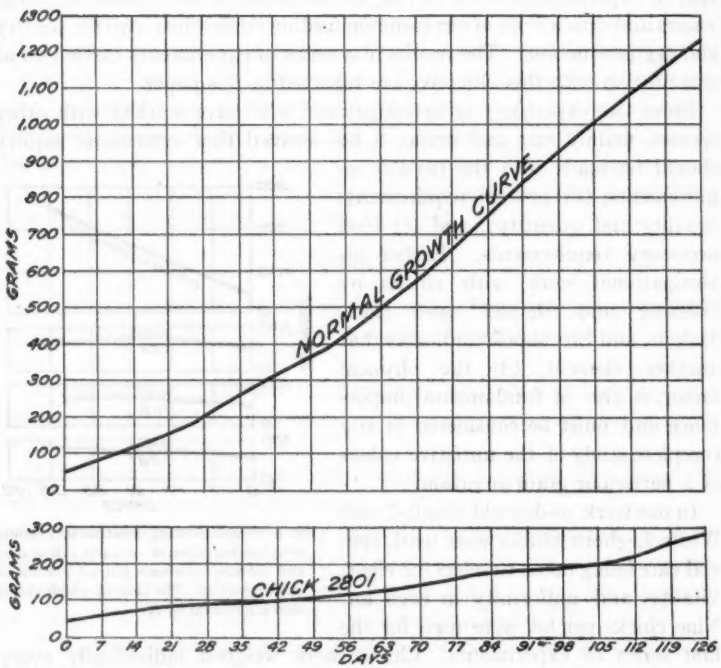


FIG. 2.—Graph showing slow but continuous growth of chick in lot 211, fed ration of 95 parts yellow corn and 5 parts ash mixture

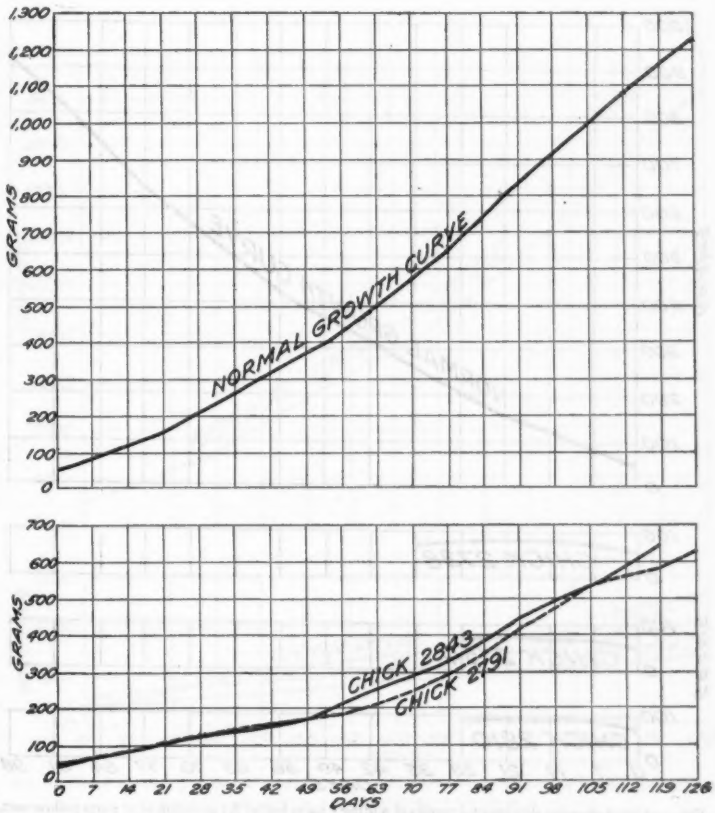


FIG. 3.—Graph showing growth of chicks in lot 213, fed ration of 80 parts yellow corn, 15 parts casein, and 5 parts ash mixture.

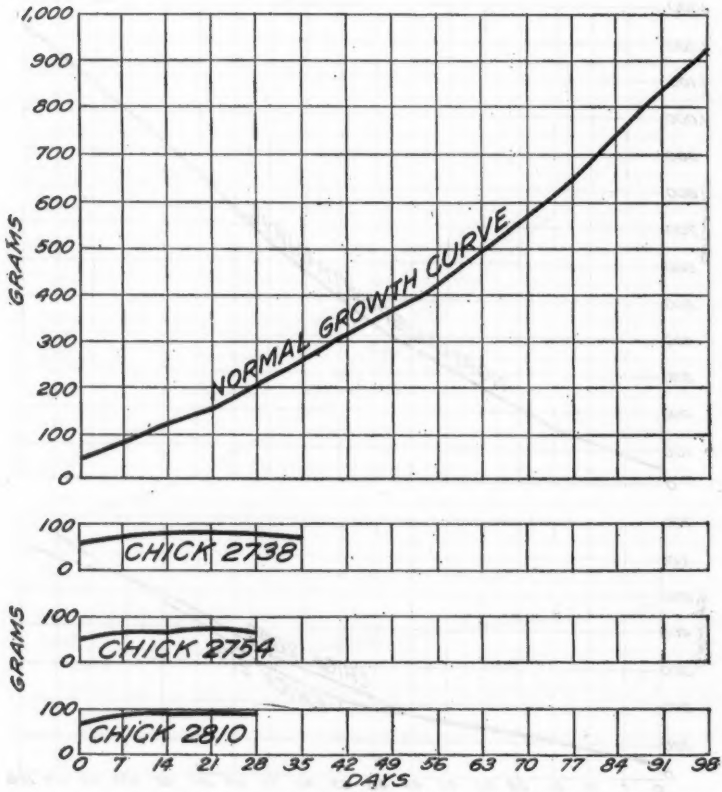


FIG. 4.—Graph showing detrimental results of adding 5 parts butter fat to ration of 65 parts yellow corn, 15 parts casein, 5 parts ash mixture, and 10 parts starch for chicks in lot 215.

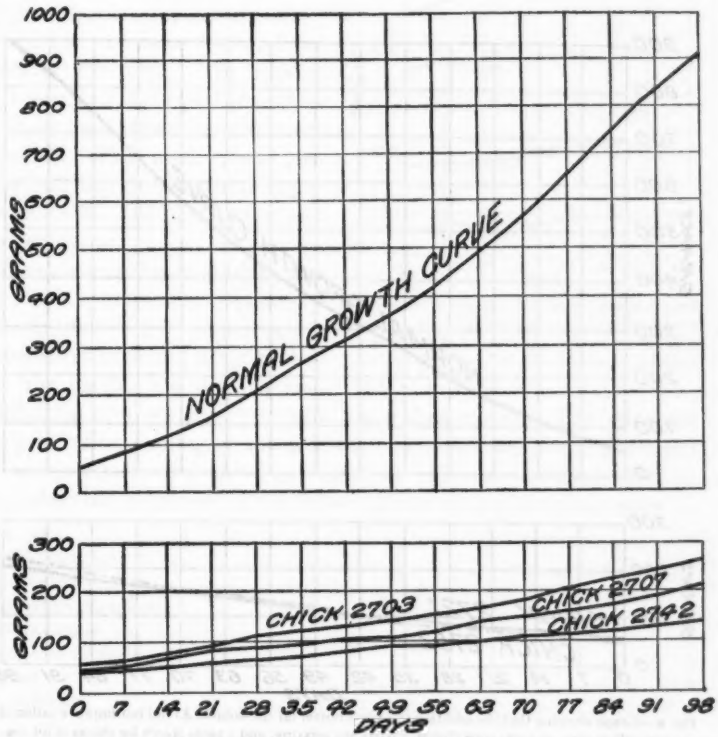


FIG. 5.—Graph showing that the addition of 20 parts corn gluten did not improve ration of 65 parts yellow corn, 5 parts ash mixture, and 10 parts starch for chicks of lot 208.

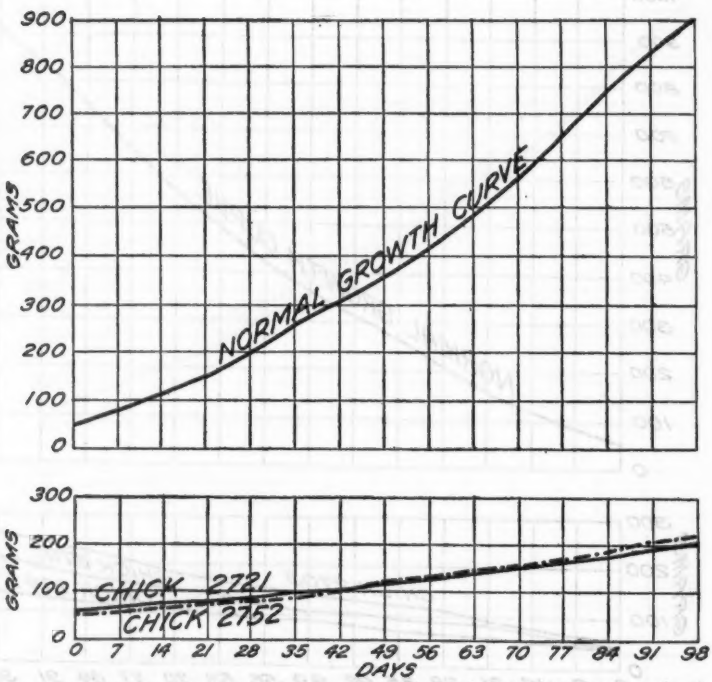


FIG. 6.—Graph showing that the addition of 5 parts butter fat (fat-soluble A) did not improve ration of 5 parts yellow corn, 20 parts corn gluten, 5 parts ash mixture, and 5 parts starch for chicks of lot 209.

consumption of each lot were also obtained. Clean wood shavings were used for litter, and each lot was confined to a pen 2 by 8 feet in size until the chicks were 8 weeks old, when the near normal lots were given a yard 4 by 8 feet in size.

Methods of feeding are known to have considerable influence on the efficiency of a ration; so a standard policy was established of dividing the ration into two parts—the scratch or coarse feed and the mash or fine feed. The rations were mixed so that equal quantities of mash and scratch feeds were provided. The mash feed contained all the supplemental ingredients, such as ash, butter fat, and purified casein.

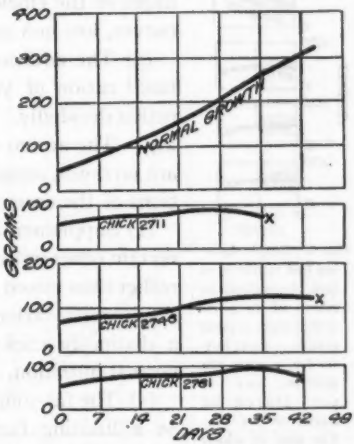


FIG. 7.—Graph showing that the addition of 15 parts soybean meal did not improve ration of 80 parts yellow corn and 5 parts ash mixture for chicks of lot 210. The time at which chicks died is indicated by *X*.

The casein used in the rations was purified by extracting repeatedly with distilled water slightly acidified with acetic acid. After extraction the casein was drained and dried in an air oven at 70° to 100° C. and then ground. The butter fat was purified by melting at 40° in a water bath and was then centrifuged to remove ash, casein, and other material. The ash mixture[1] used in our rations was composed of the following ingredients, parts by weight:

Bone ash	50
Calcium carbonate	14
Sodium chlorid	15
Dipotassium phosphate	10
Calcium lactate	5
Magnesium sulphate	3
Sulphur	2
Iron sulphate	1

The results of our inquiries may briefly be summarized as follows:

(1) Yellow corn (maize) is deficient in several of the essential qualities necessary for the complete nutrition of growing chicks. A deficiency in the ash content of the yellow corn kernel is no doubt responsible for the early failure of baby chicks when restricted to a ration of corn alone. Supplementing the corn kernel with 5 per cent of a complete ash mixture improved the ration so as to enable very slow but persistent growth.

[1] PHILIPS, A. G., CARR, R. H., and KENNARD, D. C. MEAT SCRAPS VERSUS SOY-BEAN PROTEINS AS A SUPPLEMENT TO CORN FOR GROWING CHICKS. *In* Jour Agr. Research, v. 18, no. 7, p. 391–398, 1 fig., pl. 50. 1920

(2) Yellow corn is deficient in quality and quantity of protein required for normal growth of chicks. The addition of more corn protein by including corn gluten in the ration did not markedly improve the efficiency of the ration. Compare growth curves, lots 208 and 211.

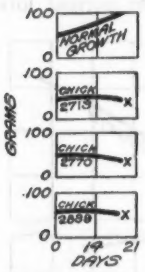


FIG. 8.—Graph showing bad results from lack of roughage in ration of 65 parts yellow corn, 15 parts gelatin, 5 parts butter fat, 5 parts ash mixture, and 10 parts starch for chicks of lot 217. The time at which chick died is indicated by *X*.

(3) The addition of 15 per cent purified casein to a basal ration of yellow corn and ash did improve the ration decidedly. Compare growth curves, lots 211 and 213. The amino acid deficiencies of the corn proteins are no doubt supplemented by the amino acid contributions of the casein.

(4) Supplementing the basal yellow corn ration with certain other proteins, egg albumen, and gelatin, lowered rather than raised the efficiency of the ration. The poor results with rations 217 and 218 were probably due to a distinctly sticky physical quality which prevented normal nutrition.

(5) The fat-soluble food accessory does not appear to be a limiting factor in a yellow corn diet for baby chicks. The addition of butter fat to a yellow corn, casein, and ash ration (lot 215) did not improve but rather lowered the efficiency of the ration. A slight change in the physical condition of the ration may explain this lowered efficiency, though it is more probable that the butter fat addition temporarily stimulated growth so that the supply of some other essential accessory was exhausted earlier than would have been the case had the butter fat been omitted. It is apparent at least that the failure of chicks on ration 215 was not due to fat-soluble A starvation.

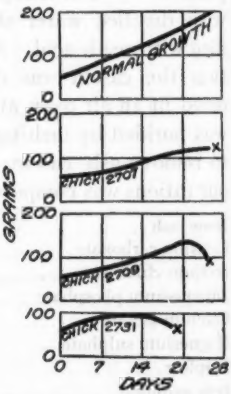


FIG. 9.—Graph showing that because proper physical quality was lacking the addition of 15 parts egg albumen did not improve ration of 65 parts yellow corn, 5 parts butter fat, 5 parts ash mixture, and 10 parts starch for chicks of lot 218. The time at which chicks died is indicated by *X*.

(6) Green feeds make certain very valuable contributions to a ration for growing chicks. The addition of wheat greens to a yellow corn, casein, and ash ration effected a decided improvement in the efficiency of the ration. An excess of the wheat greens was offered, and subsequent observations indicate that about 5 per cent (dry matter basis) of this kind of green food are consumed when offered regularly in excess. The helpful influence of the wheat greens may have been due to, first, an improvement of the physical condition of the ration; second, a food accessory con-

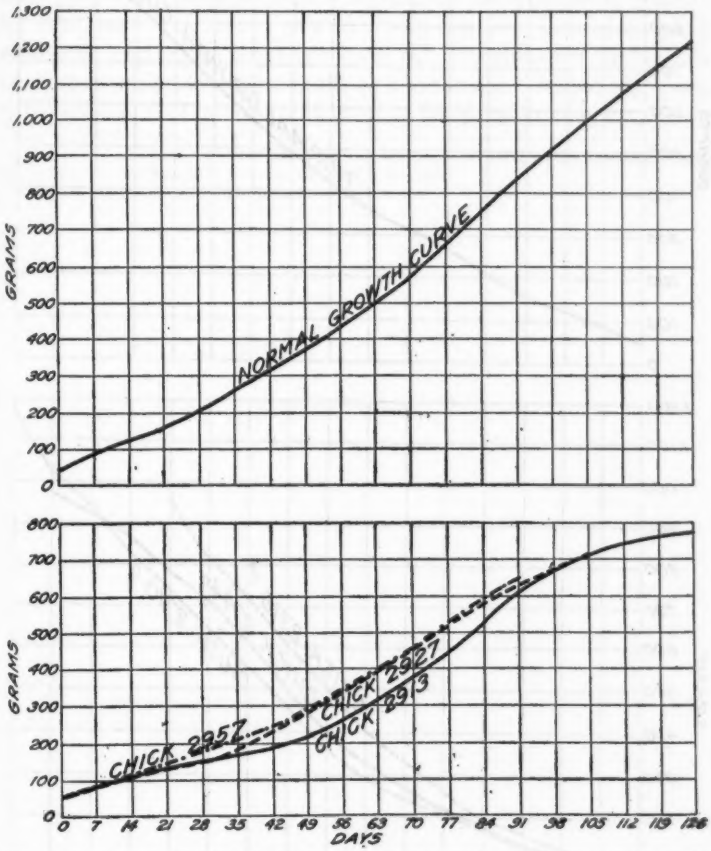


FIG. 10.—Graph showing that the addition of excess of wheat greens improved ration of 80 parts yellow corn, 15 parts casein, and 5 parts ash mixture for chicks of lot 227.

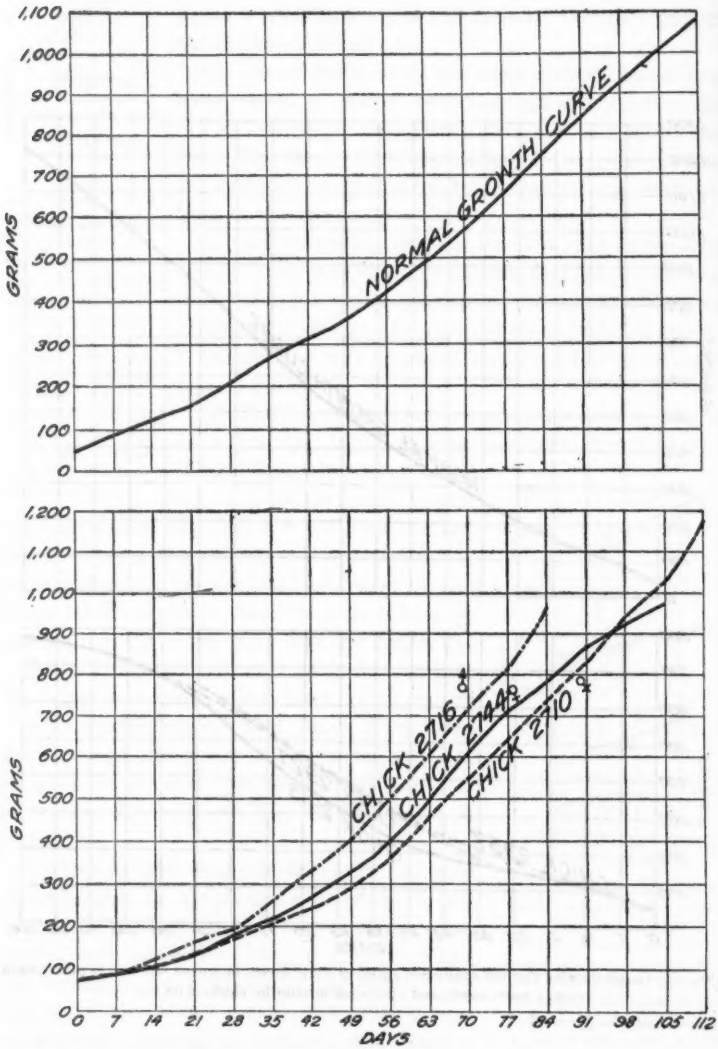


FIG. 11.—Graph showing normal growth produced by ration of 65 parts wheat, 15 parts casein, 5 parts butter fat, 5 parts ash mixture, 10 parts starch, and excess of wheat greens for chicks of lot 205. Birds were kept on this ration until they produced eggs, at age of about 200 days.

tribution; third, an increased food consumption, due to a stimulatory effect on the appetite. A series of experiments to shed further light on the specific contributions which green feeds make to the ration is in progress at this time.

(7) It is possible to raise to normal maturity chicks confined to a small pen. Drummond [1] reports great difficulty in rearing chicks in confinement, and other investigators have noted some of the problems, especially leg weakness.[2] Our lot 205 grew to normal maturity, some of the pullets producing eggs when about 200 days old, though never having more range than was provided in a yard 4 by 8 feet in size. Ration 205, though not synthetic, is of interest because of its comparative simplicity.

[1] DRUMMOND, Jack Cecil. OBSERVATIONS UPON THE GROWTH OF YOUNG CHICKENS UNDER LABORATORY CONDITIONS. *In* Biochem. Jour., v. 10, no. 1, p. 77–88, 1 pl. 1916.

[2] HART, E. B., HALPIN, J. G., and STEENBOCK, H. OP. CIT.

AECIAL STAGE OF THE ORANGE LEAFRUST OF WHEAT, PUCCINIA TRITICINA ERIKS.[1]

By H. S. JACKSON, *Chief in Botany*, and E. B. MAINS, *Associate Botanist, Purdue University Agricultural Experiment Station, and Agents, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

This paper presents, in part, the results of a study of the leafrusts of wheat, rye, barley, corn, and related grasses which was begun in 1918. One of the important phases of this investigation is the determination of the aecial relationships of the various races or species included in the collective species, *Puccinia Clematidis* (DC.) Lagerh. (*P. Agropyri* Ellis and Ev.), and other closely related forms. While a number of the rusts of this group which occur on wild grasses have been connected with aecia, their host limitations and interrelations are not well understood. This study is especially important in the case of the leafrust of wheat, *P. triticina* Eriks. So long as the aecial stage of this species was unknown, little progress could be made in developing our knowledge with reference to its origin, development, spread, and relation to other rusts. The results of the investigation of the aecial relationship of this rust are presented in the following pages.

HISTORICAL REVIEW

Three rusts are known to attack wheat: the black or stemrust, *Puccinia graminis* Pers.; the stripe or yellow rust, *P. glumarum* (Schmidt) Eriks. and Henn.; and the orange or leafrust, *P. triticina*. Of these the stemrust is the only one for which the aecial stage has been determined. This rust was shown by De Bary to have its aecial stage on *Berberis vulgaris* L., and this relationship has since been demonstrated repeatedly by a number of workers in various parts of the world. The discovery of the place of *Aecidium Berberidis* Pers. in the life cycle of *P. graminis* caused De Bary (*4, p. 207–211*)[3] to turn his attention to the study of other grass rusts having incomplete life cycles. This resulted in the discovery that *P. rubigo-vera* (DC.) Wint. (*P. straminis* Fckl.) on rye was connected with aecia on *Anchusa officinalis* and *Anchusa arvensis*. Sowings made with teliospores from rye resulted in the production of

[1] Published with the approval of the Director as a contribution from the Department of Botany, Purdue University Agricultural Experiment Station. Cooperative investigation between the Purdue University Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] The writers wish to acknowledge their indebtedness to various pathologists throughout the country for aid in obtaining material for the cultural studies upon which this paper is based, and to Mr. Forest Fuller, temporary culture assistant during the spring of 1919, and Mr. Emile Mardfin and Miss Florence M. Smith, Scientific Assistants, Office of Cereal Investigations, Bureau of Plant Industry, for assistance in carrying out the cultural investigations.

[3] Reference is made by number (italic) to "Literature cited," p. 170–171.

aecia on Anchusa, and when sowings were made with aeciospores from Anchusa, uredinia on rye were developed. Sowings of basidiospores from rye upon *Berberis vulgaris* Hol., *Rhamnus Frangula*, *Rhamus cathartica*, *Ranunculus acris*, *Ranunculus bulbosus*, *Taraxacum officinalis*, and *Urtica dioica* were without result. Nielsen (*20*, *p. 37*) 10 years later reported obtaining infection with aeciospores from *Anchusa officinalis* on both rye and wheat. Plowright (*21*, *p. 168*) states that in the fall of 1885 he obtained aecia upon *Anchusa arvensis* by placing wheat straw rusted with *P. rubigo-vera* near that host.

At the time this work was carried out the name *Puccinia rubigo-vera* was used for the leafrusts of wheat, rye, and barley, as well as for similar grass rusts having globoid urediniospores and long covered telia. Eriksson and Henning (*11*, *p. 197–203, 257–259*) separated this species into two—*Puccinia glumarum*, the stripe rust, and *Puccinia dispersa* Eriks., the brown rust. Under the latter they included the rust of wheat as well as that of rye. The rust of wheat, however, was considered as a forma specialis, *Tritici*, of *Puccinia dispersa*. As the leafrust of rye had been shown by De Bary (*4*) to be connected with aecia on Anchusa, Eriksson (*10*, *p. 254–257*) sought for the same connection for the leafrust of wheat. His sowings of basidiospores from wheat upon *Anchusa officinalis* and *A. arvensis*, however, produced no infection, as was also the case when aeciospores from Anchusa were sown on wheat. No results were obtained when basidiospores were sown on *Nonnea rosea*, *Myosotis arvensis*, *M. alpestris*, *Symphytum asperrimum* and *Pulmonaria officinalis*, species of Boraginaceae related to Anchusa upon which unconnected aecia were known to occur. As a result of these cultures, Eriksson (*10*, *p. 270*) concluded that the orange leafrust of wheat was a distinct species and gave it the name, *Puccinia triticina*.

Klebahn (*17*, *p. 85–86*; *18*, *p. 246*) made rather extensive cultures in an endeavor to discover the aecial host of *Puccinia triticina*. Besides sowing aeciospores of *Aecidium Anchusae* Eriks. and Henn. on wheat he made sowings of basidiospores on *Anchusa arvensis* and *Anchusa officinalis* without result. Sowings of basidiospores also were made without success upon *Triticum vulgare*, *Ranunculus acer*, *Ranunculus asiaticus*, *Ranunculus auricomus*, *Ranunculus bulbosus*, *Ranunculus Ficaria*, *Ranunculus flammula*, *Ranunculus lanuginosus*, *Ranunculus repens*, *Anemone ranunculoides*, *Aconitum Lycoctonum*, *Aconitum Napellus*, *Berberis vulgaris*, *Nasturtium* sp., *Barbaraea vulgaris*, *Melandryum album*, *Coronaria flos-cuculi*, *Agrostemma Githago*, *Rhamnus cathartica*, *Lythrum Salicaria*, *Ribes Grossularia*, *Aegopodium Podagraria*, *Pastinaca sativa*, *Valeriana dioica*, *Knautia arvensis*, *Tussilago Farfara*, *Taraxacum officinale*, *Centaurea Cyanus*, *Achillea Ptarmica*, *Campanula rotundifolia*, *Ligustrum vulgare*, *Phillyrea* sp., *Echium vulgare*, *Lithospermum purpureo-coeruleum*, *Myosotis* sp., *Symphytum officinale*, *Glechoma hederacea*, *Prunella vulgaris*,

Rumex acetosa, and *Urtica dioica*. These results substantiate those obtained by Eriksson and indicate that the positive results reported by Nielsen (*20*) and Plowright (*21*) were probably due to a mixture of rusts or of hosts.

The failure to obtain infection on Boraginaceous hosts has influenced other workers to turn their attention to other families in a search for the aecial hosts. Arthur (*1, v. 9, p. 304*), largely as a result of morphological studies, reached the conclusion that *Puccinia triticina* was best considered a race of *P. Agropyri*, and upon this basis Arthur and Fromme (*3, p. 333–337*) have placed it in the collective species *Dicaeoma Clematidis* (DC.) Arth. Several races of this collective species had been shown by workers in Europe and America to go to species of Clematis. Arthur thought that the aecial host might be either *Clematis flammula* or *C. vitalba* as these were the only common species of Clematis found in the wheat-growing regions of southern Europe, northern Africa, and western Asia, a region which at that time was considered as the probable home of the original wild wheat. His culture with wintered telia of the leaf-rust of wheat on *C. flammula*, however, was unsuccessful.

According to Butler (*6, p. 15*) Cunningham and Prain (*9*) considered that there was considerable ground for believing that an Aecidium on *Launaea asplenifolia*, one of the Cichoriaceae, was the aecial stage of *Puccinia triticina*, as it was found throughout the greater part of the wheat-growing area of India. Butler, however, sowed aeciospores from this host upon wheat without obtaining infection.

These unsuccessful attempts to demonstrate an aecial stage for *Puccinia triticina* have resulted in the development of the idea that the aecial stage of this rust has been lost and that it is able to maintain itself without one. In this connection a number of important facts have been established and a number of interesting hypotheses proposed. It has been shown by Bolley (*5, p. 13–14*), Hitchcock and Carleton (*15, p. 1–2*), Carleton (*8, p. 21–22*), and others that in certain regions, *P. triticina* is able to overwinter by means of its uredinal mycelium and that no aecial host is necessary for the maintenance of this species. This does not appear, however, to be true for all regions where *P. triticina* is abundant (*6, p. 11*). A number of suggestions have been made to explain the yearly appearance of the rust in regions where the urediniospores or uredinial mycelium does not overwinter. It was considered possible that spores may be carried from other regions by the wind. The mycoplasm theory of seed transmissal has also been put forward as a possible explanation. Whatever may be the merits of these hypotheses, they have resulted in recent years in directing attention away from a search for the aecial host of this species.

BASIS OF CULTURAL INVESTIGATIONS

A study of *Puccinia triticina* in comparison with other grass rusts with long covered telia shows that it can not be readily separated morphologically from the leafrust of rye. The separation of this form as a species was made by Eriksson (*10*) because he obtained only slight infection on rye with urediniospores and was not able to obtain infection on Anchusa with basidiospores and because the teliospores germinated in the spring, while those of the rye rust germinated in the fall. The close morphological similarity, however, furnished considerable grounds for the assumption that the aecial host of leafrust of wheat was likely to be some species of Boraginaceae other than Anchusa, especially as another rust of this type, *Puccinia bromina* Eriks., has since been found to have its aecia on the Boraginaceous hosts *Symphytum officinale* and *Pulmonaria montana*, with very weak development of aecia on Anchusa (*19, p. 182–202*). Unfinished investigations now being conducted in this laboratory strongly indicate that in America certain grass rusts having aecia on Boraginaceous hosts are very similar to the leafrust of wheat and rye. For these reasons it was considered desirable to test as many Boraginaceous hosts as were available, as possible aecial hosts for the leafrust of wheat.

There is, however, still another group of grass rusts very similar to the orange leafrust of wheat to which Arthur (*1, v. 9, p. 304*) has called attention. This group has aecia upon various Ranunculaceous hosts and includes forms which have been separated from time to time, according to their aecial connection, together with slight morphological variation, into a number of species, including *Puccinia persistens* Plowr., *P. perplexans* Plowr., *P. Agropyri*, and *P. alternans* Arth. The writers felt from the beginning that the greatest possibility of success in the search for the aecial stage was to study thoroughly the genera of this family on which aecia were known to occur.

The idea that *Puccinia triticina* has lost its ability to develop an aecial stage through long propagation by urediniospores, while admittedly possible, was not considered to be fully substantiated.

CULTURES MADE IN 1919

With these considerations in mind rather extensive sowings were made in the spring of 1919 upon a considerable number of species of the families Ranunculaceae and Boraginaceae and the closely related family Hydrophyllaceae. For this purpose, 20 collections of telia of *Puccinia triticina* were obtained from various sections of the country during the summer and fall of 1918 and placed outdoors to winter. Early in March these began to germinate. Ten of the 20 collections gave good germination and were sown upon various species of the above-named families and upon *Ornithogalum umbellatum L.*, *Impatiens* sp., and *Camassia esculenta* (Ker.) Robins. (*Quamasia hyacinthina*). The results obtained are given in Table I.

TABLE I.—*Data obtained in 1919 from sowing teliospores of Puccinia triticina, from 10 different localities, on various host plants, mostly of the families Ranunculaceae and Boraginaceae* [a]

Host inoculated.	No. 118 (Okla.).	No. 218 (Ala.).	No. 418 (Tenn.).	No. 618 (Ga.).	No. 718 (Ga.).	No. 818 (S. C.).	No. 918 (Ind.).	No. 3518 (Wis.).	No. 3818 (Wis.).	No. 4518 (Wis.).
Aconitum Fischeri Reich			–							
Aconitum Napellus L.		–								–
Actaea spicata L.	–				–					
Anemone canadensis L.	–		–					–	–	–
Anemone cylindrica Gray	–			–		–	–	–		
Anemone japonica Sieb. & Zucc.		–			–		–			
Anemone sp.	–									
Aquilegia alpina L.		–	–							–
Aquilegia canadensis L.	–			–		–		–		–
Aquilegia chrysantha Gray			–		–				–	–
Aquilegia glandulosa Fisch.	–					–				
Aquilegia Skinneri Hook.	–					–				
Aquilegia vulgaris L.	–		–		–				–	
Aquilegia sp.			–							
Cimicifuga racemosa (L.) Nutt.	–					–				
Clematis Douglasii Hook.	–					–				
Clematis Fremontii Wats.								–		
Clematis heracleaefolia DC.										–
Clematis ligusticifolia Nutt.			–					–		
Clematis orientalis L.			–		–		–			
Clematis recta L.	–	–	–		–					–
Clematis virginiana L.	–		–		–	–		–	–	–
Clematis sp.			–		–		–		–	
Delphinium ajacis L.					–					
Delphinium "*Belladonna*." (Hort.)				–	–					
Delphinium consolida L.					–					
Delphinium Geyeri Greene			–		–					
Echium vulgare L.	–	–								
Hepatica sp.	–					–				
Hydrophyllum appendiculatum Michx.	–				–					–
Impatiens sp.	–				–	–	–	–	–	–
Mertensia virginica (L.) Link		–	–	–						
Myosotis palustris Lam.		–	–							
Myosotis scorpoides L.	–	–								–
Ornithogalum umbellatum L.	–					–	–			
Phacelia tanacetifolia Benth.					–					
Phacelia Purshii Buckl.	–				–		–			
Camassia esculenta (Ker.) Robins.	–									
Ranunculus aconitifolius L.		–	–						–	–
Ranunculus acris L.				–			–			
Ranunculus repens L.	–						–			
Thalictrum angustifolium L.							[b] °–			
Thalictrum aquilegifolium L.							[b] °–			
Thalictrum dioicum L.	–		–		–		–			–
Thalictrum minus L.							–			
Thalictrum polygamum Muhl.								–		
Thalictrum sp.									–	
Trollius europeus L.			–							–

[a] – No infection.
° Pycnia produced.
[b] Two sowings were made. Pycnia were produced from only one sowing, no result being obtained from the other.

The telial collections used in the cultures were all obtained from *Triticum aestivum* (*T. vulgare*) in the following localities:

118, from Stillwater, Okla., collected by J. D. Moore.
218, from Flint, Ala., collected by McClellan.
418, from Tennessee, collected by W. T. Evans.
618, from Carrolton, Ga., collected by R. O. Burns.
718, from Carrolton, Ga., collected by R. O. Burns.

818, from Anderson, S. C., collected by R. O. Burns.
918, from La Fayette, Ind., collected by E. H. Toole.
3518, from Menah, Wis., collected by E. H. Toole.
3818, from Wisconsin, collected by E. H. Toole.
4518, from Superior, Wisconsin, collected by E. H. Toole.

Negative results were obtained on all but two species of the hosts used. The collection from La Fayette, Ind. (No. 918), gave infection upon *Thalictrum angustifolium* and *T. aquilegifolium*, producing, however, only pycnia. It was impossible to carry this study further in 1919, as the above results were not obtained until late in the spring.

The failure of aecia to develop from the two successful infections could be explained on either of two hypotheses. The conditions in the greenhouse may have been unfavorable, or the species of Thalictrum used may have been resistant. In either case, however, these results were interpreted as indicating that the aecial host of the leafrust of wheat was some species of Thalictrum. There was considerable basis for this assumption. All of the culture studies being carried on in this laboratory with the related rusts, occurring on wild grasses, and having aecia on members of the family Ranunculaceae, have indicated that while a given race may develop aecia on several species in one host genus with varying degrees of virulence it will not go to species of more than one genus. The rusts of this group show a very high degree of specialization. The two species of Thalictrum on which infection was obtained were foreign species, while the North American species, *Thalictrum dioicum* and *T. polygamum*, were not infected. On this account it was thought that the susceptible aecial hosts for the leafrust of wheat probably were foreign species of Thalictrum. As the leafrust of wheat presumably is an introduced form, as explained in the following pages, this would be expected, and on that basis the species of Thalictrum should be western Asiatic or eastern European, corresponding to the region in which wheat is believed to have originated.

CULTURES MADE IN 1920

In preparation for cultural studies for the spring of 1920 an effort was made during the summer and fall of 1919 to obtain as many species of Thalictrum as possible. It was impossible to obtain material from foreign botanical gardens in time to be of use, and the best that could be done was to secure such species of Thalictrum as were carried by nurserymen in this country, together with such native species as could be obtained through collectors in various parts of the United States. As a result 14 species were brought together. An appeal was also made to the plant pathologists in the various agricultural experiment stations throughout the country for aid in securing telial material of the leafrust of wheat. A very gratifying response to this appeal was made, and in this way 80 collections of telia were obtained and placed out to overwinter. Of these, 51 collections germinated in the spring of 1920 and were sown. The number of collections was so great that it was not possible to sow

them on as large a number of species as was done in 1919, and attention was devoted mainly to sowing upon species of Thalictrum. Of the 51 collections used 9 were sown upon as many Thalictrum species as possible in order to determine the relative susceptibility of these species. The results are given in Table II.

TABLE II.—*Data obtained in 1920 from sowing teliospores of Puccinia triticina from nine different localities on various species of Thalictrum*[a]

Host inoculated.	Laboratory No.	No. 5619 (Ga.).	No. 6019 (N. C.).	No. 7219 (N. C.).	No. 7819 (Tenn.).	No. 8019 (Mich.).	No. 8719 (Miss.).	No. 11619 (Pa.).	No. 12519 (Idaho).	No. 15119 (Nev.)
Thalictrum angustifolium L.	6	[b] 1	o	o	.....	—	o	[b] 1	—	—
Thalictrum aquilegifolium L.	5	o	—	—	.....	.....	—o	o	.....	[b] 1
Thalictrum aquilegifolium L.	66	—	—	—	.....	—	—(2)	—	—	—
Thalictrum dasycarpum Fisch. and Lall.	65	.....	—	—	.....	.....	.....	o	—	o
Thalictrum Delavayi Franchet	56	.....	o	1	o1	—	1	o	1	o
Thalictrum dioicum L.	16	1	o	.....	.....	—	—	o	.....	.....
Thalictrum flavum L.	53	1	o	1	—	1	1	1	1	1
Thalictrum minus L.	17	—	—	o	[b] 1	—	—	[b] 1	.....	.....
Thalictrum minus adiantifolium	63	[b] 1	—(2)	— (2)	o	—	— (3)	—	o	—
Thalictrum occidentale Gray	115	—	—	—	—	.....	.....	—	.....	.....
Thalictrum polycarpum S. Wats.	114	.....	.....	.....	.....	.....	.....	[b] 1	[b] 1	.....
Thalictrum polygamum Muhl.	19	—	— (2)	—	o	—	— (2)	—	.....	—
Thalictrum sp.	98	o	—	o1	.....	o	1—	—	o	o
Thalictrum sp.	55	.....	1	1	1	—	.....	1	1	—

[a] — No infection.
o Pycnia only produced.
1 Aecia following pycnia.
A numeral in parenthesis following the sign indicates the number of times the results were obtained.
[b] Although aecia were produced, the infection was weak.

The following is a list of the sources of the telial material used in the cultures:

5619, from Athens, Ga., collected by C. A. Ludwig.
6019, from W. Raleigh, N. C., collected by Ludwig and Wolf.
7219, from Hickory, N. C., collected by C. A. Ludwig.
7819, from Tennessee, collected by C. A. Ludwig.
8019, from Coldwater, Mich., collected by B. W. Mains.
8719, from Canton, Miss., collected by C. A. Ludwig.
11619, from State College, Pa., collected by J. T. Adams.
12519, from Moscow, Idaho, collected by C. W. Hungerford.
15119, from Reno, Nev., collected by G. R. Hoerner.

In addition to the sowings indicated in Table II, culture 8719 was sown upon *Aquilegia glandulosa* Fisch., *A. olympica* Boiss., *Clematis heraclaefolia* DC., *C. paniculata* Thunb., *C. recta* L., and *Ranunculus acris* L., all without infection.

An examination of Table II shows that 12 out of the 14 species of Thalictrum were infected, *Thalictrum occidentale* and *T. aquilegifolium* apparantly being immune. The species on which infection occurred showed varying degrees of susceptibility. *Thalictrum dasycarpum* and

T. polygamum gave mostly negative results or the occasional production of pycnia. *T. angustifolium*, *T. aquilegifolium* (5), *T. minus*, *T. minus adiantifolium*, and *T. polycarpum* showed occasionally a weak development of aecia, but usually only pycnia developed or no infection occurred. *T. dioicum*, in one case, showed a moderate development of aecia; in all other cases only pycnia developed, or no infection resulted. *T. Delavayi* and *T.* sp. (98) (Pl. 21, A, B) showed fairly vigorous infection, accompanied in most cases by more or less hypertrophy and usually by well-developed aecia. *T. flavum* (Pl. 21, C) and *T.* sp. (55) (Pl. 21, D) showed a very vigorous infection accompanied usually by pronounced hypertrophy of the infected leaf and petiole tissue and practically always with the production of well-developed aecia.

An attempt has been made to check the determination of the species of Thalictrum used in these studies, but this has been difficult because a number of them have produced neither flowers nor fruit, and the leaf characters in this genus are in most cases extremely variable. Specimens of most of the species have been sent to Mr. S. F. Blake, of the Bureau of Plant Industry, Washington, D. C., who has kindly compared them with specimens in the United States National Herbarium and has given his opinion as to the identity of our material. The following list gives the species used above, their sources, and native distribution as accurately as they could be determined. The accession number of this laboratory follows the name of each species.

Thalictrum angustifolium L. (6). Source: Seed from Brooklyn Botanic Garden. Distribution: Central Europe and Asia Minor.

Thalictrum aquilegifolium L. (5 and 66). Source: Bobbink and Atkins Nursery Co. Distribution: Europe, Middle and Northern Asia. (No. 66 was purchased for *T. paniculatum*.)

Thalictrum dasycarpum Fisch. and Lall. (65). Source: Department of Botany, Michigan Agricultural College. Distribution: Northern and central United States and southern Canada.

Thalictrum Delavayi Franchet (56). Source: Farr Nursery Co. Distribution: Western China.

Thalictrum dioicum L. (16). Source: LaFayette, Ind. Distribution: Eastern United States.

Thalictrum flavum L. (53). Source: Farr Nursery Co. Distribution: Europe, Western Asia, and Asia Minor.

Thalictrum minus L. (17). Source: An American nursery. Distribution: Europe, Asia, and eastern and southern Africa.

Thalictrum minus adiantifolium (63). Source: Seed from Brooklyn Botanic Garden. Distribution: See *T. minus*.

Thalictrum occidentale Gray (115). Source: Corvallis, Oreg. Distribution: Mountains, California to British Columbia.

Thalictrum polycarpum S. Wats. (114). Source: Berkeley, Calif. Distribution: California.

Thalictrum polygamum Muhl. 19. Source: Ithaca, N. Y. Distribution: Eastern United States.

Thalictrum sp. (98). Source: Palisade Nursery Co. Distribution: Exotic.

Thalictrum sp. (55). Source: Farr Nursery Co. Distribution: Exotic.

From the data presented above it is evident that a number of species of Thalictrum are susceptible hosts for *Puccinia triticina*. As far as the host determinations are at all certain, the evidence would indicate that the most susceptible hosts are from western Asia and eastern Europe, and doubtless in this region other species will be found of as great or greater susceptibility.

The remaining collections of telia showing good germination were sown on one or more of the susceptible species of Thalictrum in order to determine how uniformly *Puccinia triticina* from the United States would go to Thalictrum. Table III gives the results of these cultures.

TABLE III.—*Data obtained in 1920 from sowing teliospores of Puccinia triticina, from many different localities, on four especially susceptible species of Thalictrum* [a]

Number and source of telia.	*T.* sp. (55).	*T. Delavayi* (56).	*T. flavum* (53).	*T.* sp. (98)
5119 Pa.		I		
11719 Pa.				o
9819 W. Va.				I—
6319 N. C.		I		o
6419 N. C.		o		
7319 N. C.		o		
5819 S. C.				I—
5019 Ga.				I—(2)
4119 Ala.				o
4519 Ala.			I	
4419 Tenn.[b]				—
17419 Tenn.		—		I
6519 Ky.		o		
4819 Ind.		I		
5319 Ind.				I
10119 Ind.		I		
10419 Ill.				I
12419 Minn.				Io
3619 Iowa				o
9219 Mo.				o
9319 Mo.		o		
9519 Mo.				o
9619 Mo.	I			
3819 La.				o
6719 Tex.				I
7019 Tex.[c]				o
3219 Ariz.				I
6119 Calif.		o		
12219 Wash.				o
16519 Wash.		I		I
19919 Wash.	I			
13919 Oreg.				o

[a] — No infection.
o Pycnia produced.
I Aecia following pycnia.
A numeral (in parenthesis) following the sign indicates the number of times the results were obtained.
[b] Sown also on *T. angustifolium*, producing pycnia, and on *T. aquilegifolium* without results.
[c] Sown also on *T. dasycarpum* without results.

Source of telial material used in cultures:

5119, from York, Pa., collected by F. D. Kern.
11719, from Bradford County, Pa., collected by E. T. Nixon.
9819, from Morgantown, W. Va., collected by N. J. Giddings.
6319, from Statesville, N. C., collected by C. A. Ludwig.
6419, from Statesville, N. C., collected by C. A. Ludwig.
7319, from Biltmore, N. C., collected by C. A. Ludwig.
5819, from Clemson College, S. C., collected by C. A. Ludwig.
5019, from Tifton, Ga., collected by C. A. Ludwig.
4119, from Bay Minette, Ala., collected by C. A. Ludwig.
4519, from Auburn, Ala., collected by C. A. Ludwig.
4419, from Union City, Tenn., collected by Carl Kurtzweil.
17419, from Johnson City, Tenn., collected by C. A. Ludwig.
6519, from Lexington, Ky., collected by R. S. Kirby.
4819, from Mount Vernon, Ind., collected by E. B. Mains.
5319, from Washington County, Ind., collected by H. S. Jackson.
10119, from La Fayette, Ind., collected by E. B. Mains.
10419, from Bloomington, Ill., collected by Koehler and Toole.
12419, from Wasioja, Minn., collected by G. W. Martin.
3619, from Ames, Iowa, collected by I. E. Melhus.
9319, from Columbia, Mo., collected by W. E. Maneval.
9419, from Columbia, Mo., collected by W. E. Maneval.
9519, from Columbia, Mo., collected by W. E. Maneval.
9619, from Columbia, Mo., collected by W. E. Maneval.
3819, from Baton Rouge, La., collected by Thiel and Ludwig.
6719, from Dallas, Tex., collected by W. H. Ballamy.
7019, from San Antonio, Tex., collected by R. S. Kirby.
3219, from Yuma, Ariz., collected by L. Y. Leonard.
6119, from Chico, Calif., collected by R. M. Kelia.
12219, from Dayton, Wash., collected by J. W. Hotson.
16519, from Colton, Wash., collected by J. W. Hotson.
19919, from Puyallup, Wash., collected by G. R. Hoerner.
13919, from Oregon, collected by G. R. Hoerner.

The data in this table, taken with those in Table II, show that *Puccinia triticina* from Pennsylvania, West Virginia, North Carolina, South Carolina, Georgia, Alabama, Mississippi, Tennessee, Kentucky, Indiana, Michigan, Illinois, Minnesota, Iowa, Missouri, Louisiana, Texas, Arizona, California, Washington, Oregon, Idaho, and Nevada gave positive results when sown upon Thalictrum.

The following collections showed some germination but produced no infection when sown on Thalictrum:

11819, from Hopkinsville, Ky., collected by Carl Kurtzweil.
9419, from Columbia, Mo., collected by W. E. Maneval.
3919, from Fayetteville, Ark., collected by H. R. Rosen.
5419, from Memphis, Tenn., collected by A. F. Thiel.

7519, from Southampton, N. Y., collected by H. S. Jackson.
8319, from Rocky Ford, Colo., collected by J. G. Leach.
10019, from Buffalo, Minn., collected by G. W. Martin.
10519, from Plainview, Nebr., collected by H. W. Thurston.
11019, from Vermillion, Minn., collected by G. W. Martin.
11219, from Newark, Del., collected by T. F. Manns.

Besides the above, the following collections were wintered, but no germinating teliospores were found, and in consequence they were not sown.

1419, from Santa Rosa, Calif., collected by H. S. Jackson.
3319, from Sonora, Mexico, near Yuma, Ariz., collected by L. Y. Leonard.
3419, from St. Louis, Mo., collected by E. B. Mains.
3719, from Jackson, Tenn., collected by Kurtzweil and Thiel.
4019, from Corvallis, Oreg., collected by G. R. Hoerner.
5519, from St. Paul, Minn., collected by A. F. Thiel.
6619, from Hiawatha, Kans., collected by W. H. Ballamy.
6819, from Marshall, Mo., collected by R. S. Kirby.
6919, from Guthrie, Okla., collected by R. S. Kirby.
7119, from Wellington, Mich., collected by G. H. Coons.
7919, from Nashville, Tenn., collected by C. A. Ludwig.
9119, from Madison, Wis., collected by E. B. Mains.
10819, from Manhattan, Kans., collected by L. E. Melchers.
11119, from Toledo, Iowa, collected by I. E. Melhus.
11919, from Fort Collins, Colo., collected by J. G. Leach.
12019, from East Lansing, Mich., collected by Acelia M. Leach.
12119, from East Lansing, Mich., collected by Acelia M. Leach.
12319, from Pullman, Wash., collected by F. D. Heald.
13819, from Fort Collins, Colo., collected by J. G. Leach.
21019, from Moscow, Idaho, collected by G. R. Hoerner.
25519, from Murfreesboro, Tenn., collected by Carl Kurtzweil.
25719, from Clarksville, Tenn., collected by Carl Kurtzweil.

The accompanying map (fig. 1) shows the source of collections used in the work together with the results obtained with them at La Fayette, Ind. This map shows that material from the States of Pennsylvania, West Virginia, Indiana, Illinois, North Carolina, South Carolina, Georgia, Alabama, Mississippi, Louisiana, and Texas gave germination uniformly and infected Thalictrum in all cases. A region represented by the States of Colorado, Oklahoma, Arkansas, Kansas, Nebraska, Minnesota, Iowa, and Missouri, with one arm running through Wisconsin into Michigan and another through Tennessee into Kentucky, gave material which usually did not germinate or, if germination was obtained, produced infection on Thalictrum in only a few cases. Whether this situation indicates the presence of another strain of the leafrust having different characteristics as regards its viability and power to infect Thalictrum, or whether it means that the season or climate was of such a nature that

teliospores of a low vitality were produced, remains for future investigation to decide. Telial material from the Pacific coast, while not viable in a number of cases, produced infection on Thalictrum in all cases where germination was obtained.

The aecia produced from the following telial collections were sown back upon wheat:

4519, from Auburn, Ala.
4819, from Mount Vernon, Ind.
5019, from Tifton, Ga.
5619, from Athens, Ga.
5819, from Clemson College, S. C.
6019, from W. Raleigh, N. C.
6319, from Statesville, N. C.

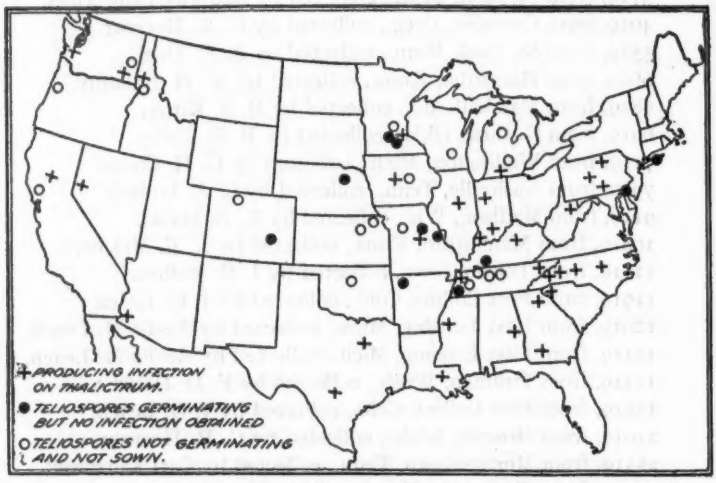

+ PRODUCING INFECTION ON THALICTRUM.
● TELIOSPORES GERMINATING BUT NO INFECTION OBTAINED
○ TELIOSPORES NOT GERMINATING AND NOT SOWN.

FIG. 1.—Map showing results obtained at LaFayette, Ind., with leafrust material collected in different parts of the United States.

7219, from Hickory, N. C.
7819, from Tennessee.
8019, from Coldwater, Mich.
8719, from Canton, Miss.
9619, from Columbia, Mo.
9819, from Morgantown, W. Va.
10419, from Bloomington, Ill.
11619, from State College, Pa.
12419, from Wasioja, Minn.
12519, from Moscow, Idaho.
15119, from Reno, Nev.
16519, from Colton, Wash.
17419, from Johnson City, Tenn.

These were each sown upon the variety of wheat known as Dawson Golden Chaff, and in all cases positive infection was obtained followed by the development of uredinia which were typical of *Puccinia triticina*.

Sowings of aeciospores also were made upon a number of grasses. Aecia which were produced from telia obtained from Hickory, N. C., Canton, Miss., and Moscow, Idaho, were used and Table IV gives the results.

TABLE IV.—*Results obtained in 1920 from sowing the aeciospores of Puccinia triticina produced from telia obtained in three different areas, on wheat and related grasses*

Host inoculated.	Number and source of aecia.		
	No. 7219 (N. C.).	No. 8719 (Miss.).	No. 12519 (Idaho).
Arrhenatherum elatius (L.) Mert & Koch	.....	.....	–
Agropyron caninum (L.) Beauv	–	–	.....
Agropyon cristatum Beauv	.....	.....	–
Agropyron desertorum Schult	.....	.....	–
Agropyron inerme (Schribn. & Sm.) Rydb	.....	.....	–
Agropyron repens (L.) Beauv	–	–	.....
Agropyron tenerum Vasey	–	–	.....
Elymus australis Schribn. & Ball	–	–	.....
Elymus canadensis L	–	–	.....
Elymus glaucus Buckl	–	–	.....
Elymus triticoides Buckl	.....	.....	–
Elymus virginicus L	–	–	.....
Hordeum caespitosum Schribn	.....	.....	–
Hordeum gussoneanum Parl	.....	.....	–
Hordeum jubatum L	–	–	–
Hordeum pusillum Nutt	–	–	.....
Hordeum murinum L	.....	.....	–
Hordeum vulgare L	.....	–	.....
Hystrix Hystrix (L.) Millsp	–	–	.....
Notholcus lanatus (L.) Nash	.....	.....	–
Secale cereale L	.....	† One uredinium.	.....
Sitanion Hystrix (Nutt.) J. G. Sm	.....	–	.....
Triticum aegilops Beauv	.....	.....	† Few.
Triticum aestivum L	† Many.	† Many.	† Many.

– No infection. † Uredinia produced.

Except for the one uredinium produced on *Secale cereale*, *Triticum aestivum* and *T. aegilops* were the only species infected

DESCRIPTION OF AECIA

The following description has been drawn from the aecia obtained in the cultures discussed above.

Pycnia amphigenous, mostly epiphyllous, numerous, crowded upon more or less swollen reddish brown to yellowish areas 2 to 15 mm. in diameter, conspicuous, subepidermal, honey-yellow, globoid or flattened globoid, 80 to 145 μ broad by 80 to 130 μ high; ostiolar filaments 95 to 190 μ long, agglutinated to form a prominent, broad column.

Aecia hypophyllous, crowded in more or less swollen, gall-like, reddish brown or yellowish areas 2 to 15 mm. in diameter, cupulate or short cylindric, 0.2 to 0.6 mm. in diameter, up to 0.5 mm. high; peridium white or yellowish, the margin erose or somewhat lacerate, recurved; peridial cells oblong or somewhat rhomboidal in longitudinal radial section, 14 to 19 by 18 to 29 μ, abutted or slightly overlapping, the outer wall 6 to 7 μ thick, transversely striate, the inner wall thinner 2 to 3 μ, very coarsely verrucose; aeciospores angularly globoid or ellipsoid, 16 to 20 by 16 to 26 μ; wall colorless, thin, 1 μ or less, very closely and finely verrucose.

The pycnia and aecia usually were produced in definite galls or swellings. These galls apparently were formed by the excessive enlargement of the cells of the infected areas, especially those of the mesophyll. When infection took place in the young, rapidly developing tissue of the petiole, galls developed (Pl. 21, D) which were 10 or 15 times as large as the normal petiole. A very noticeable odor, resembling that of the hyacinth, was often detected as the pycnia reached maturity.

GENERAL DISCUSSION OF RESULTS

The discovery that species of Thalictrum are the aecial hosts for *Puccinia triticina* goes to support Arthur's contention (*1, v. 9, p. 304*) that the leafrust of wheat is closely related to grass rusts of the type of *Puccinia Agropyri*, having aecia on species of the family Ranunculaceae. A number of cultures have been made with rusts of this type, connecting them with various species of Thalictrum. Plowright, in England (*21, p. 181*), connected aecia on *Thalictrum flavum* with a rust on *Agropyron repens*. To this rust he gave the name *Puccinia persistens* Plowr. He considered *Aecidium Ranunculacearum γ Thalictri flavi* DC., and *Aecidium Thalictri flavi* (DC.) Winter as synonyms, and describes the aecia as occurring on thickened spots with aeciospores subglobose 17 to 20 by 20 to 30 μ. Fischer (*12, p. 57–63*), in Switzerland, cultured a rust from *Poa nemoralis* var. *firmula* on *Thalictrum minus*, *T. aquilegifolium* and *T. foetidum*. On account of the morphological similarity, he concluded that his material belonged to *Puccinia persistens*, although he made no cultures on either *Agropyron repens* or *T. flavum*. He describes the aecia (*13, p. 347–349*) as having peridial cells with the outer wall 4.5 to 13.5 μ thick and the inner 2 to 6 μ and aeciospores 10 to 21 μ broad and up to 28 μ long. An examination of Sydow's Uredineen No. 725, issued as *Puccinia persistens* on *T. aquilegifolium*, shows the following measurements: Peridial cells, 18 to 23 by 21 to 26 μ; outer wall, 7 to 9 μ; inner, 3 to 5 μ; aeciospores, 16 to 19 by 19 to 26 μ.

Juel (*16, p. 411*), in Sweden, made cultures connecting aecia on *Thalictrum alpinum* with a rust on *Agrostis borealis* and *Anthoxanthum odoratum*. To this rust he gave the name *Puccinia borealis* Juel, and considered *Aecidium thalictri* Grev. as a synonym. His description follows: Pycnia not present; aecia not causing hypertrophy of host tissue; aecio-

spores about 13 μ in diameter. The Sydows (*23, p. 718–719*) give the measurement of the aeciospores as 13 to 18 μ in diameter or 13 to 16 by 18 to 20 μ and note that no swellings are produced on the leaves of the host. An examination of Eriksson's Fungi Parasitici Scandinavici 432a, collected by Juel in Norway, gives the following measurements: Peridial cells, 16 to 19 by 19 to 29 μ; the outer wall, 10 μ; the inner, 3 to 4 μ; aeciospores 14 to 16 by 16 to 21 μ.

Rostrup (*22, p. 269–273*), in Denmark, obtained infection with aeciospores from *Thalictrum minus* on *Elymus arenarius* and considered the rust to be *Puccinia Elymi* Westendorp. The writers have seen neither description nor material of these aecia.

The Sydows (*23, p. 827*) mention that Lindroth in Finland connected an aecidium on *Thalictrum majus* with a rust on *Agropyron caninum*. No description or material of this connection is available for study.

In North America a number of connections have been established by the cultures of Arthur and of Fraser. Arthur (*1, v. 1, p. 248–249*) reports culturing a rust found associated with aecia on *Thalictrum sparsiflorum* from *Bromus Porteri* to *T. dioicum*. To this he gave the name *Puccinia alternans*. He describes the aecia as having peridial cells 21 to 29 μ long with the outer wall 9 to 12 μ thick and the inner 5 to 7 μ and with aeciospores 15 to 20 by 17 to 24 μ. A number of other species of Thalictrum are given as hosts.

Arthur (*1, v. 2, p. 226*) also reports obtaining infection from telia on Agropyron resulting in aecia on *Thalictrum alpinum* but not on *T. dioicum*. This material he considered as belonging in *Puccinia obliterata* Arth., which he had previously shown as having aecia on Aquilegia. A study of the material obtained by this culture shows little or no hypertrophy of the host tissue. The peridial cells measure 16 to 21 by 24 to 32 μ, having the outer wall 7 to 9 μ thick and the inner 3 to 5 μ. The aeciospores measure 14 to 18 by 18 to 23 μ.

Still another connection was obtained by Arthur (*1, v. 8, p. 132–133*) when he cultured a rust on *Festuca Thurberi* to *Thalictrum dioicum*, producing aecia. To this he later (*2, p. 113*) gave the name *Puccinia Cockerelliana* Bethel. He gives the peridial cells as 16 to 23 by 27 to 36 μ with the outer wall 6 to 8 μ and the inner 2 to 3 μ and aeciospores 18 to 24 by 20 to 29 μ with a wall 1.5 to 2.5 μ thick. The natural host for the aecia is given as *T. Fendleri*.

Fraser (*14, p. 131–133*) reports sowing aeciospores from *Thalictrum dasycarpum* on *Elymus canadensis*, *E. virginicus*, *Agropyron tenerum*, *A. Richardsonii*, *Hordeum jubatum*, *Triticum vulgare*, and *Bromus ciliatus*, obtaining infection on *E. canadensis*, *E. virginicus*, *H. jubatum*, and *B. ciliatus*. When, however, the rust obtained upon *B. ciliatus* was sown on *E. virginicus*, *A. tenerum*, *A. Smithii*, *A. repens*, and *H. jubatum* no infection was obtained on these species. From these results Fraser concludes that two strains of *Puccinia Agropyri* Ellis and E.,

were present in the aecial material on Thalictrum which he used for the culture. A study of the aecia used in these cultures shows the dimensions of the peridial cells to be 15 to 19 by 23 to 29 μ with the outer wall 7 to 10 μ and the inner 3 μ thick and the aeciospores 14 to 19 by 19 to 23 μ in diameter.

A comparison of the foregoing description of the aecia of *Puccinia triticina* with the measurements given for the various grass rust aecia on Thalictrum shows surprisingly little variation. The aecia of *P. Cockerelliana* show the greatest difference, having larger peridial cells and somewhat larger aeciospores with much thicker walls than the aecia of *P. triticina*. Slightly smaller aeciospores occur in *P. borealis* and *P. obliterata*, and the aecial infection causes little or no hypertrophy of the host. The remaining aecia differ mainly in slightly thicker walls of the peridial cells.

It is evident that *Puccinia triticina* is closely related to *P. persistens*. Whether the name *Aecidium Thalictri-flavi* (DC.) Wint. should apply to the aecial stage of the former is a question which can not be answered with the available information. De Candolle (*7, p. 97*) described *A. Ranunculacearum* for aecia occurring on the family Ranunculaceae and as a variety of this gives *Thalictri-flavi* without further description. Winter (*24, p. 269*) raises this variety to specific rank and gives a description which, however, could apply to the aecia of either rust. As both *P. triticina* and *P. persistens* are common rusts throughout Europe, there is no way of determining definitely to what aecia the name was applied beyond the fact that they were on *Thalictrum flavum*. As it has been shown that at least some aecia on that host in England belong to *P. persistens* the name *A. Thalictri-flavi* should be retained for the present as a synonym of that species, at least until aecia can be found in Europe upon *T. flavum* which will produce the leafrust of wheat.

Upon their grass hosts these rusts present a somewhat greater variation. They all have uredinia with globoid or ellipsoid urediniospores with a varying number of scattered pores, usually more than six, and with few or no paraphyses. The telia are long, covered by the epidermis, usually with more or less stroma present, and the teliospores are cylindric, more or less flattened at the apex, and with a very short pedicel. *Puccinia Cockerelliana* differs most noticeably from *P. triticina* in that the teliospores are much longer and the telia do not remain entirely covered by the epidermis at maturity. *P. Elymi* differs especially in the thicker and darker walls of the urediniospore and in the longer teliospores which are often many-celled. *P. alternans*, *P. borealis*, *P. obliterata*, and *P. persistens* differ but little, mostly in the tinting of the urediniospore wall and a slight variation in pore number.

Although the morphological differences between *Puccinia triticina* and the related rusts discussed above are not great, their biologic specializa-

tion to their hosts is very pronounced. This appears to hold true for the aecial as well as the grass hosts. *P. Cockerelliana* and *P. alternans* go to *Thalictrum dioicum*, and *P. Elymi* to *T. minus*, as aecial hosts, neither of which is a favorable host for *P. triticina*. The rust of *T. dasycarpum*, used by Fraser (*14*) in his cultures, is on a host which was not infected by *P. triticina*. *P. borealis* and *P. obliterata* on *T. alpinum* offer no comparison, as *P. triticina* was not sown on that host. *P. persistens*, as cultured by Fischer on *T. minus* and *T. aquilegifolium*, is upon species of Thalictrum unfavorable for *P. triticina*, while *P. persistens* as originally cultured by Plowright upon *T. flavum* is on the most congenial host for the leafrust of wheat. It is very probable that Plowright and Fischer were working with two distinct biologic strains. Although *T. flavum* appears to be a favorable host for both *P. persistens* and *P. triticina*, and these two rusts are very similar in their morphology, the inability of the latter to infect *Agropyron repens* shows that it is biologically distinct from the former. A study is being made of the relationship of *P. triticina* to grass hosts other than wheat. From the data now at hand, it would appear that, in addition to the grasses listed in Table IV, species of Bromus, Festuca, Agrostis, Poa, and Anthoxanthum are immune from the leafrust of wheat. These results indicate that, as far as its telial host is concerned, *P. triticina* also is biologically distinct from other grass rusts having aecia on Thalictrum.

A similar situation exists in the relationship of *Puccinia triticina* to rusts having aecia upon species of other genera of the Ranunculaceae. Slight morphological differences, such as urediniospore size, wall color, and pore number, exist among the different races producing aecia upon species of such genera as Actaea, Anemone, Clematis, Delphinium, etc. A similar, or perhaps greater, biologic specialization is also to be found among these races. The importance of these morphological characters and biological differences which occur among the members of this group can not be fully determined at present on account of our comparatively limited knowledge of but few races. Any final interpretation must await further study of a greater number of such races. On the basis of our present knowledge, the disposition of *P. triticina* must depend largely upon the species concept held. In Europe there is a tendency among certain students of the rusts to consider as species those rusts showing distinct biologic specialization regardless of the absence of morphological difference. In this country, on the other hand, the general tendency is to include in a single species all closely related forms having but little difference in their morphology. Forms limited to a definite host, or hosts, are considered as races of such species. On the former basis, *P. triticina* would be considered a distinct species comparable to *P. Elymi*, *P. Agropyri*, *P. persistens*, etc., while with the latter concept it would be united with all or part of these, each being considered a race of a

collective species to be designated, according to the limitations of the species concept held and the system of nomenclature used, as *P. Agropyri* E. and E. (*1, v. 9, p. 304*), *P. Clematidis* (DC.) Lagerh., or *Dicaeoma Clematidis* (DC.) Arth. (*3, p. 333–337*).

The close biological specialization of *Puccinia triticina* to wheat is of considerable significance with respect to the bearing it has upon the possible origin of this rust and of wheat itself. Since wheat is an introduced plant, it is logical to assume that a rust showing such close biological specialization to it is also introduced and of foreign origin.

It is generally recognized among students of the rusts that a high degree of host specialization must have been acquired in certain groups of species at a very early stage in the evolutionary history of this group of fungi. It is also recognized that the host is the most important factor in the evolution of highly specialized parasitic fungi. As the higher plants have gradually developed during geological times, their rust parasites have developed with them. It therefore appears reasonable to assume that *Puccinia triticina*, which shows such a high degree of specialization to wheat at the present time, had its origin as a distinct strain comparatively early in the development of the group of grasses from which our cultivated wheats have originated. The original distribution of the rust presumably would coincide with the distribution of the ancestral wheats.

A study of the relative susceptibility of various species of Thalictrum to infection by this rust is of interest in this connection. The four most susceptible species of Thalictrum encountered in this investigation are all of foreign origin. The most susceptible of our native North American species, *Thalictrum dioicum*, does not compare in susceptibility with these four foreign species—*T. flavum*, *T. Delavayi*, *T.* sp. 55, and *T.* sp. 98—but is comparable to the resistant foreign species such as *T. minus*. That these foreign susceptible species of Thalictrum are also to be considered as indicating a foreign origin of the rust would appear to follow if the nature of aecial infection is considered. Heteroecious rusts in most cases infect their aecial hosts only for a comparatively short period of time while the teliospores are germinating in the spring. The infection produced, not being able to propagate itself upon such hosts, causes little or no damage, and they are in most cases soon able to outgrow it. On this account it is hardly to be expected that a natural selection of resistant strains of aecial hosts takes place in nature comparable to that which occurs where the host is killed or prevented from producing seed. Should this occur in heteroecious rusts which are not able to survive adverse conditions in winter or summer by means of urediniospores, such a selection would be fatal to the rust itself. For this reason the susceptibility of the aecial hosts of *P. triticina* may be taken as indicative of its origin. It is true that susceptibility of a host species does not necessarily indicate that such a species was a native host of the rust nor does resistance

of some one species denote that the rust is not to be found in the habitat of such a resistant species, for susceptibility or resistance is not dependent upon the presence or absence of the rust but may develop with the species in any region. It is regarded as significant, however, that of the species of Thalictrum tested the most susceptible are exotic. This fact, taken with the foreign origin of wheat itself, is confirmative of the foreign origin of the rust.

The native habitats of two of these species of Thalictrum are known with some degree of certainty. *Thalictrum flavum* is found throughout Europe, western Asia, and Asia Minor. *T. Delavayi* is given by the Index Kewensis [1] as occurring in western China, probably indicating a distribution in the little-known mountainous regions of Tibet and Chinese Turkestan. These two species, taken together, would therefore indicate as the most probable original distribution a region in which the two Thalictrum species may border or overlap, such as that of southwestern Asia. Such an origin would indicate a like origin for wheat itself, which, we believe, would agree with the latest theories advanced as to the original home of wheat.

Concerning the occurrence and distribution of the aecia of *Puccinia triticina* but little can be said with the data at hand. It is also probable that the aecial stage occurs, and probably assumes greater importance, in other regions than it may in either Europe or North America, where the rust is known to overwinter in its uredinial stage. Thus in such countries as India, where Butler has shown there is no oversummering of the rust, the Thalictrum species of the foothills may be of importance in starting the rust the next season. The question of the rôle which the aecia of the leafrust of wheat plays in its life history and distribution must, however, be left for future research to solve. Whether native species of Thalictrum serve as aecial hosts in North America and, if so, whether they serve as important factors in the development of the leafrust of wheat and whether there is more than one race of the leafrust, as indicated by the results obtained from the Great Plains area, or whether these results were due to other causes, such as climatic or seasonal effects weakening the vitality of the teliospores, are all questions on which further investigation is planned. Other species of Thalictrum from foreign botanic gardens also will be studied in regard to their susceptibility to the orange leafrust of wheat.

SUMMARY

(1) The aecial stage of *Puccinia triticina* has been produced in greenhouse cultures upon several species of Thalictrum.

(2) The various species of Thalictrum show varying degrees of susceptibility to the rust. *Thalictrum occidentale* was apparently immune. Upon *T. dasycarpum* and *T. polygamum* an occasional devlopment of

[1] INDEX KEWENSIS PLANTARUM PHANEROGAMARUM. SUPPLEMENTUM PRIMUM . . . CONFECERUNT THEOPHILUS DURAND ET B. DAYDON JACKSON. p. 423. Bruxellis 1901–06.

pycnia took place. When *T. angustifolium*, *T. aquilegifolium*, *T. dioicum*, *T. minus*, *T. minus adiantifolium*, and *T. polycarpum* were inoculated usually only pycnia resulted, with an occasional weak development of aecia, while in other cases no infection occurred. Two undetermined species of Thalictrum, as well as *T. Delavayi* and *T. flavum*, when inoculated, showed a vigorous development of aecia, increasing in susceptibility in the order named.

(3) *Puccinia triticina* is apparently limited to species of the genus Thalictrum, no infection being obtained upon species of Aconitum, Actaea, Anemone, Aquilegia, Cimicifuga, Clematis, Delphinium, Echium, Hepatica, Hydrophyllum, Impatiens, Mertensia, Myosotis, Ornithogalum, Phacelia, Camassia, Ranunculus, or Trollius.

(4) On account of the morphology and host relationships, *Puccinia triticina* is considered to be very closely related to *P. persistens*, *P. borealis*, *P. alternans*, *P. obliterata*, *P. Elymi*, and *P. Agropyri*, but is separable from these rusts by its sharp biologic limitation to wheat.

(5) *Puccinia triticina* is considered to be of foreign origin, because wheat, for which it shows close specialization, is an introduced host, and because the most susceptible species of Thalictrum which serve as aecial hosts also are exotic.

LITERATURE CITED

(1) Arthur, Joseph Charles.
1909–17. cultures of uredineae in 1908, 1909, 1915, 1916 and 1917. *In* Mycologia v. 1, no. 6, p. 225–256; v. 2, no. 5, p. 213–240; v. 8, no. 3, p. 125–141; v. 9, no. 5, p. 294–312.

(2) ———
1919. new species of uredineae—xi. *In* Bul. Torrey Bot. Club, v. 46, no. 4, p. 107–125.

(3) ——— and Fromme, Fred. Denton.
1920. dicaeoma on poaceae. *In* North American Flora, v. 7, pt. 4–5, p. 269–341.

(4) Bary, Anton de.
1866. neue untersuchungen über uredineen. *In* Monatsber. K. Preuss. Akad. Wiss. Berlin, 1866, p. 205–215, 1 pl.

(5) Bolley, H. L.
1889. wheat rust. Ind. Agr. Exp. Sta. Bul. 26, 19 p., 9 fig.

(6) Butler, E. J., and Hayman, J. M.
1906. indian wheat rusts. *In* Mem. Dept. Agr. India, Bot. Ser., v. 1, no. 2, p. 1–52, 1 fig., 5 pl.(1–4 col.).

(7) Candolle, A. P. de.
1815. flore française... t. 5 (v. 6). Paris.

(8) Carleton, Mark Alfred.
1899. cereal rusts of the united states. a physiological investigation. U. S. Dept. Agr. Div. Veg. Physiol. and Path. Bul. 16, 74 p., 1 fig., 4 col. pl. Bibliography, p. 70–73.

(9) Cunningham, D. D., and Prain, D.
1896. a note on indian wheat-rusts. *In* Rec. Bot. Survey India, v. 1, no. 7, p. 99–124.

(10) ERIKSSON, Jakob.
1899. NOUVELLES ÉTUDES SUR LA ROUILLE BRUNE DES CÉRÉALES. *In* Ann. Sci. Nat. Bot. sér. 8, t. 9, no. 2/4, p. 241–288, pl. 11–13 (col.). Littérature citée, p. 286–287.
(11) ——— and HENNING, Ernst.
1894. DIE HAUPTRESULTATE EINER NEUEN UNTERSUCHUNG ÜBER DIE GETREIDEROSTE. *In* Ztschr. Pflanzenkrank., Bd. 4, p. 66–73, 140–142, 197–203, 257–262.
(12) FISCHER, Eduard.
1898. ENTWICKLUNGSGESCHICHTLICHE UNTERSUCHUNGEN ÜBER ROSTPILZE... x, 120 p., 16 fig., 2 pl. (Beiträge zur Kryptogamenflora der Schweiz, Bd. 1, Heft. 1.)
(13) ———
1904. DIE UREDINEEN DER SCHWEIZ. xciv, 590 p., 342 fig. Bern. Literaturverzeichniss, p. 558–576. (Beiträge zur Kryptogamenflora der Schweiz Bd. 2, Heft. 2.)
(14) FRASER, W. P.
1919. CULTURES OF HETEROECIOUS RUSTS IN 1918. *In* Mycologia, v. 11, no. 3, p. 129–133.
(15) HITCHCOCK, A. S., and CARLETON, M. A.
1894. SECOND REPORT ON RUSTS OF GRAIN. Kans. Agr. Exp. Sta. Bul. 46, 9 p.
(16) JUEL, H. O.
1894. MYKOLOGISCHE BEITRÄGE I. ZUR KENNTNISS EINIGER UREDINEEN AUS DEN GEBIRGSGEGENDEN SKANDINAVIENS. *In* Öfvers. K. Svenska Vetensk. Akad. Förhandl., Årg. 51, no. 8, p. 409–418.
(17) KLEBAHN, Heinrich.
1900. BEITRÄGE ZUR KENNTNIS DER GETREIDEROSTE. II. *In* Ztschr. Pflanzenkrank., Bd. 10, Heft. 2, p. 70–96, 1 fig.
(18) ———
1904. DIE WIRTSWECHSELNDEN ROSTPILZE... 447 p. Berlin. Literatur, p. ix–xxxvii.
(19) MÜLLER, Fritz.
1901. BEITRÄGE ZUR KENNTNIS DER GRASROSTE. *In* Bot. Centbl., Beihefte. Bd. 10, Heft 4/5, p. 181–212, 1 fig..
(20) NIELSEN, P.
1877. BEMAERKNINGER OM NOGLE RUSTARTER, NAVNLIG OM EN GENETISK FORBINDELSE MELLEM AECIDIUM TUSSILAGINIS PERS. OG PUCCINIA POARUM N. SP. *In* Bot. Tidsskr., Raekke 3, Bd. 2, p. 26–42, 3 fig.
(21) PLOWRIGHT, Charles B.
1889. A MONOGRAPH OF THE BRITISH UREDINEAE AND USTILAGINEAE... vii, 347 p., 13 fig., 8 pl. London. Authors quoted, p. 309–315.
(22) ROSTRUP, E.
1898. ET NYT VAERTSKIFTE HOS UREDINACEERNE OG KONIDIER HOS THECAPHORA CONVOLVULI. *In* Overs. K. Danske Vidensk. Selsk. Fordhandl., 1898, No. 5, p. 269–276.
(23) SYDOW, P., and SYDOW, H.
1904. MONOGRAPHIA UREDINEARUM... v. 1. Lipsiae.
(24) WINTER, Georg.
1884. DIE PILZE DEUTSCHLANDS, OESTERREICHS UND DER SCHWEIZ. ABT. I. SCHIZOMYCETEN, SACCHAROMYCETEN UND BASIDIOMYCETEN. *In* Rabenhorst, L. Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Aufl. 2. Bd. 1, Abt. 1. Leipzig.

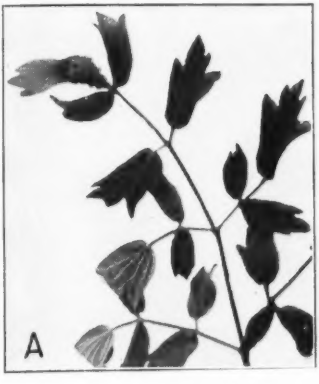
PLATE 21

A.—Infection produced upon *Thalictrum* sp. (98) inoculated with *Puccinia triticina* from Colton, Wash. (16519).

B.—Infection produced upon *Thalictrum* sp. (98) inoculated with *Puccinia triticina* from Canton, Miss. (8719).

C.—Infection produced upon *Thalictrum flavum* (53) inoculated with *Puccinia triticina* from Hickory, N. C. (7219).

D.—Infection produced upon *Thalictrum* sp. (55) inoculated with *Puccinia triticina* from Hickory, N. C. (7219).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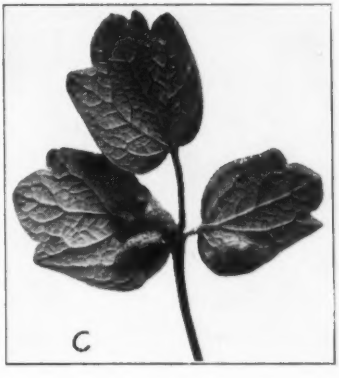
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D

A TRANSMISSIBLE MOSAIC DISEASE OF CHINESE CABBAGE, MUSTARD, AND TURNIP

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In the fall of 1919, while the writer was selecting different kinds of plants for inoculation experiments with mosaic of Irish potatoes (*Solanum tuberosum* Linn.), Dr. W. A. Orton called his attention to mottling in plants of Chinese cabbage (*Brassica pekinensis* (Lour.) Gagn.), mustard (*B. japonica* Coss.), and turnip (*B. rapa*, Linn.). The mottling resembled that of mosaic plants of other species, such as potato and tobacco. Diseased and healthy individuals were found in the same plot; the former appeared in groups in some parts of the field, suggesting an infectious character of this malady. Evidence bearing upon the nature of this disease, its symptoms, and means of transmission is presented in this paper.

SYMPTOMS

Mosaic of Chinese cabbage, mustard, and turnip produces a distinct mottling of the leaves, very similar to that of mosaic diseases of the Solanaceae. This mottling is produced by the appearance of irregular light green and dark green areas on the leaves (Pl. B; 22, D, E; 24, A, B). These light green areas usually adjoin the veins, from which they may extend so as to include a considerable area of the leaf surface between the veins. Another very common macroscopic symptom of this disease is the characteristic ruffling and distorting of the leaf surface (Pl. 24, A, B). On the raised areas the dark green patches appear. The leaf margins frequently are much more irregular than in healthy plants, causing some of the leaves to appear somewhat unsymmetrical. In addition to these common abnormalities on the leaves the entire plant may be dwarfed, and the flower stalk and number of blossoms may be considerably reduced (Pl. 22, B; 23, B).

OCCURRENCE IN THE FIELD

Since mosaic individuals appeared among Chinese cabbage, mustard, and turnip plants growing in adjoining plots, interspecific susceptibility was suggested. Furthermore, it was found that a large percentage of the plants were infested with aphids, *Myzus persicae* Sulz.,[1] one of the

[1] Identified by Dr. A. C. Baker, Entomologist, Bureau of Entomology, United States Department of Agriculture.

Journal of Agricultural Research, Washington, D. C.
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Vol. XXII, No. 3
Oct. 15, 1921
Key No. G-248

casual agents in the transmission of mosaic and leafroll of Irish potato.[1] In view of these field observations experiments on this disease were conducted in the greenhouse at Washington, D. C., during the winters of 1919–20 and 1920–21.

TRANSMISSION WITH PLANT JUICE

Chinese cabbage, mustard, and turnip plants showing mosaic mottling were taken from the field and planted in pots in the greenhouse. Only a small percentage of these mature and mosaic plants survived transplanting, so that the supply of mosaic material for inoculations was thus considerably reduced, and therefore only a small number of healthy plants were inoculated at one time.

Inoculations with juice were made by rubbing the leaves between the fingers so that considerable sections of the leaflets were crushed, apparently permitting the applied juice to be absorbed by such areas of the leaf as still remained free or partly free from mutilation. Such operations were performed chiefly upon the youngest leaves, the first applications being made when the plants had developed about five leaves. In Table I the results of these inoculations are presented.

TABLE I.—*Inoculations with juice from mosaic plants*

Variety and species inoculated.	Time of inoculation.	Source of juice.	Number of plants inoculated.	Number of plants mosaic.[a]	Percentage mosaic.
Southern Prize turnip.	Dec. 4, 1919...	Mosaic Southern Prize turnip.	9	6	67
Do.....	.....do.....	Healthy turnip.....	9	0	0
Do.....	Dec. 6, 1919...	Mosaic Green Mountain potato.	5	0	0
Mustard.....	.....do.....	.....do.....	5	0	0
Do.....	Mar. 8, 1921 Mar. 21, 1921	....do.....	6	0	0
Do.....	Mar. 9, 1921 Mar. 21, 1921	Mosaic mustard.....	8	5	63
Do.....	Jan. 15, 1921	Mosaic pe-tsai or Chinese cabbage.	5	3	60
Pe-tsai or Chinese cabbage.	Dec. 4, 1919	.....do.....	8	6	75
Do.....	.....do.....	Healthy.....	5	0	0
Do.....	Dec. 6, 1919	Mosaic Green Mountain potato.	4	0	0

[a] Date of last observation April 2, 1920 and 1921.

The data indicated in Table I disclose the fact that mosaic mottling was obtained only when juice from a mosaic plant was introduced into

[1] SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. Merrill, and HAWKINS, Lon A. INVESTIGATIONS ON THE MOSAIC DISEASE OF THE IRISH POTATO. *In* Jour. Agr. Research, v. 17, no. 6, p. 247–274, pl. A–B (col.), 25–30. 1919. Literature cited, p. 272–273.

SCHULTZ, E. S., and FOLSOM, Donald. TRANSMISSION OF THE MOSAIC DISEASE OF IRISH POTATOES. *In* Jour Agr. Research, v. 19, no. 7, p. 315–338, pl. 49–56. 1920.

SCHULTZ, E. S., and FOLSOM, Donald. LEAFROLL, NET-NECROSIS, AND SPINDLING-SPROUT OF THE IRISH POTATO. *In* Jour. Agr. Research, v. 21, no. 1, p. 47–80, pl. 1–12 1921. Literature cited, p. 78–80.

a plant of the same or a closely related species.[1] No mosaic mottling appeared on any of the cruciferous plants inoculated with juice from mosaic potato. With a more adequate supply of crucifer mosaic material and repeated applications it is probable that every plant treated would have developed mosaic mottling, such as has frequently been obtained with mosaic potato juice inoculations on the Irish potato.[2]

The first mosaic mottling was observed from 20 to 30 days after inoculation, which also corresponds very closely with the incubation period for mosaic of Irish potato. The results in Table I also disclose successful inoculations on plants in different species of Brassica. Further evidence on this interspecific infection is presented in Table II on transmission by means of aphids.

TRANSMISSION WITH APHIDS

Since aphids were found on every mosaic plant examined in the field and on account of the fact that these insects have been found to transmit mosaic of tobacco,[3] spinach blight,[4] and mosaic of potato,[5] experiments were carried on with these insects. Aphids belonging to *Myzus persicae* Sulz. were used in this investigation. These insects were originally collected from the morning-glory and transferred to healthy turnip and mustard plants on which they were cultured while confined under cages until needed for inoculation. Neither the morning-glory nor the turnip or mustard plants on which these insects fed before being transferred to mosaic Chinese cabbage and turnip developed mosaic mottling. This indicates that the morning-glory apparently was free from mosaic, at least from the type which could infect the crucifers used in this experiment.

When the healthy plants for inoculation had developed from five to eight leaves, aphids were transferred from the cultures to mosaic plants, where they were allowed to feed for a few days before they were introduced to the healthy plants. All inoculated plants also were confined in cages so as to prevent dispersal from one species to another. After the aphids had fed from 7 to 14 days on the inoculated plants they were killed by tobacco fumigation in a fumigation chamber. These plants were now allowed to grow without cages in a greenhouse where fumigation was practiced at regular intervals for the control of aphids. Since mosaic mottling developed from 12 to 30 days after these insects were killed by fumigation, mosaic mottling can not be attributed simply to the mechanical injury produced by the aphids. This fact is further

[1] GARDNER, Max W., and KENDRICK, James B. TURNIP MOSAIC. *In* Jour. Agr. Research, v. 22, no. 3, p. 123–124, 1 pl. 1921.

[2] SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. Merrill, and HAWKINS, Lon A. OP. CIT.

[3] ALLARD, H. A. THE MOSAIC DISEASE OF TOBACCO. U. S. Dept. Agr. Bul. 40, 33 p., 7 pl. 1914.

[4] MCCLINTOCK, J. A., and SMITH, Loren B. TRUE NATURE OF SPINACH-BLIGHT AND RELATION OF INSECTS TO ITS TRANSMISSION. *In* Jour. Agr. Research, v. 14, no. 1, p. 1–60, pl. A (col.), 1–11. 1918.

[5] SCHULTZ, E. S., FOLSOM, Donald, HILDEBRANDT, F. Merrill, and HAWKINS, Lon. A. OP. CIT.

confirmed by the control plants which remained free from mosaic mottling after aphids taken from healthy plants had fed upon them. The results secured from inoculation with aphids are presented in Table II.

TABLE II.—*Transmission of mosaic of mustard, pe-tsai, and turnip by means of aphids*

Variety inoculated.	Date of inoculation.	Approximate number of aphids transferred.	Source of aphids.	Number of plants inoculated.	Date of first symptoms.	Number of plants mosaic. [a]	Percentage mosaic.
Southern Prize turnip.	Jan. 12, 1920	50	Mosaic Southern Prize turnip.	2	Feb. 2, 1920	2	100
Do	Jan. 26, 1920	50	.....do.....	2	Feb. 20, 1920	2	100
Do	Jan. 12, 1920	50	Healthy turnip...	3	.....	0	0
Purple Top turnip..	Jan. 22, 1920	12	Mosaic Southern Prize turnip.	3	Feb. 14, 1921	3	100
Seven Top or South-Prize turnip.	Feb. 24, 1920	100	Mosaic mustard...	2	Mar. 20, 1920	2	100
Do	Mar. 5, 1920	100	.....do.....	2	Mar. 31, 1920	2	100
Do	Mar. 10, 1920	50	.....do.....	1	Apr. 2, 1920	1	100
Mustard	Jan. 12, 1920	50	.....do.....	1	Feb. 11, 1920	1	100
Do	Jan. 19, 1920	100	.....do.....	2	Feb. 19, 1920	2	100
Do	...do.....	100	Healthy mustard .	1	.....	0	0
Do	Jan. 26, 1920	50	Mosaic mustard...	2	.....	2	100
Do	...do.....	50	Healthy mustard..	1	.....	0	0
Do	Mar. 12, 1921	50	Mosaic mustard...	5	Apr. 2, 1921	5	100
Do	Jan. 29, 1921	25	Mosaic Southern Prize turnip.	3	Feb. 20, 1921	3	100
Do	Feb. 19, 1921	50	.....do.....	5	Mar. 25, 1921	2	40
Do	Feb. 8, 1921	95	.....do.....	2	.....	2	100
Do	Jan. 15, 1921	12	Mosaic Chinese cabbage.	3	Feb. 14, 1921	3	100
Do	Jan. 29, 1921	25	.....do.....	1	Feb. 20, 1921	1	100
Do	Feb. 19, 1921	95	.....do.....	5	Mar. 28, 1921	3	60
Do	Mar. 2, 1921	50	Mosaic Green Mountain potato.	5	.....	0	0

[a] Date of last observation, Apr. 2, 1920 and 1921.

From the data indicated in Table II it is evident that aphids transmit mosiac of the crucifers in question between different species as well as among plants of the same species, as was suggested in Table I on juice inoculations by means of rubbing. It will also be noted that the period in which the first mosaic mottling appeared corresponds very closely to that obtaining with mosiac diseases of other plants. As with the plants inoculated by rubbing, the plants inoculated by means of aphids developed the mosaic symptoms only on the younger leaves. Mosaic symptoms on the inoculated plants were like those which were observed on mosaic lots taken from the field.

Since turnips from mosaic plants taken from the field continued to produce mosaic foliage it is apparent that such plants become a source of infection if planted near susceptible varieties. Mustard seed from mosaic mustard plants apparently develop healthy seedlings. This was observed in 100 seedlings, which were grown from seed from mosaic mustard in the fall of 1920; in this test every seedling was free from mosaic mottling.

SUMMARY

From these preliminary observations and experiments it appears that the crucifers here mentioned may be added to the list of plants susceptible to mosaic, a disease whose cause has not been discovered but which can be transmitted from mosaic to healthy plants by direct transfer of juice as well as by means of aphids which apparently are very effective natural agents in the dissemination of this disease.

PLATE B

1.—Leaf from healthy turnip, control to mosaic turnip in figure 2.

2.—Leaf from mosaic turnip, mosaic induced by aphids transferred from mosaic turnip plant.

3.—Leaf from healthy mustard, control to mosaic mustard in figure 4.

4.—Leaf from mosaic mustard, mosaic produced by aphids from mosaic mustard.

3
4
2
1

A
B

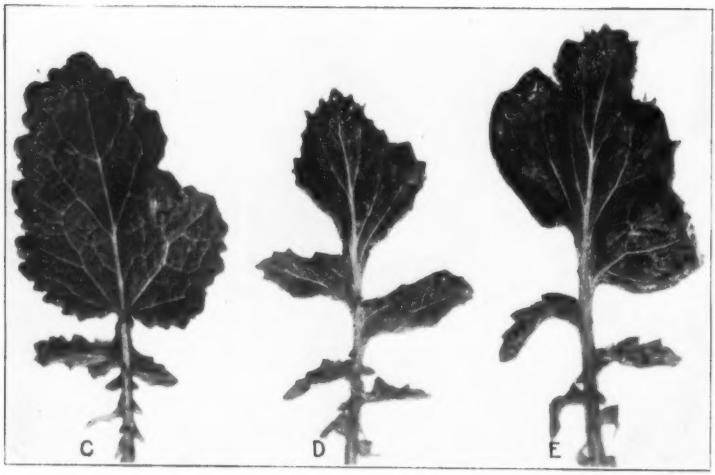
C
D
E

PLATE 22

A.—Healthy turnip plant, control to B. Aphids from healthy turnip were allowed to feed on this plant. Planted the same time as B.

B.—Mosaic on turnip plant, variety Seven Top or Southern. Mosaic mottling appeared 26 days after the introduction of aphids from a mosaic Southern turnip plant.

C.—Leaf from A, healthy.

D, E.—Two mosaic leaves from B. Mosaic mottling and ruffling apparent on the diseased leaves.

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PLATE 23

A.—Healthy mustard plant, control to B. Planted the same time as B.

B.—Mosaic on mustard plant produced by transferring aphids from mosaic mustard. Distinct mosaic mottling was noted 28 days after introduction of aphids.

A
2 M
B
1 M

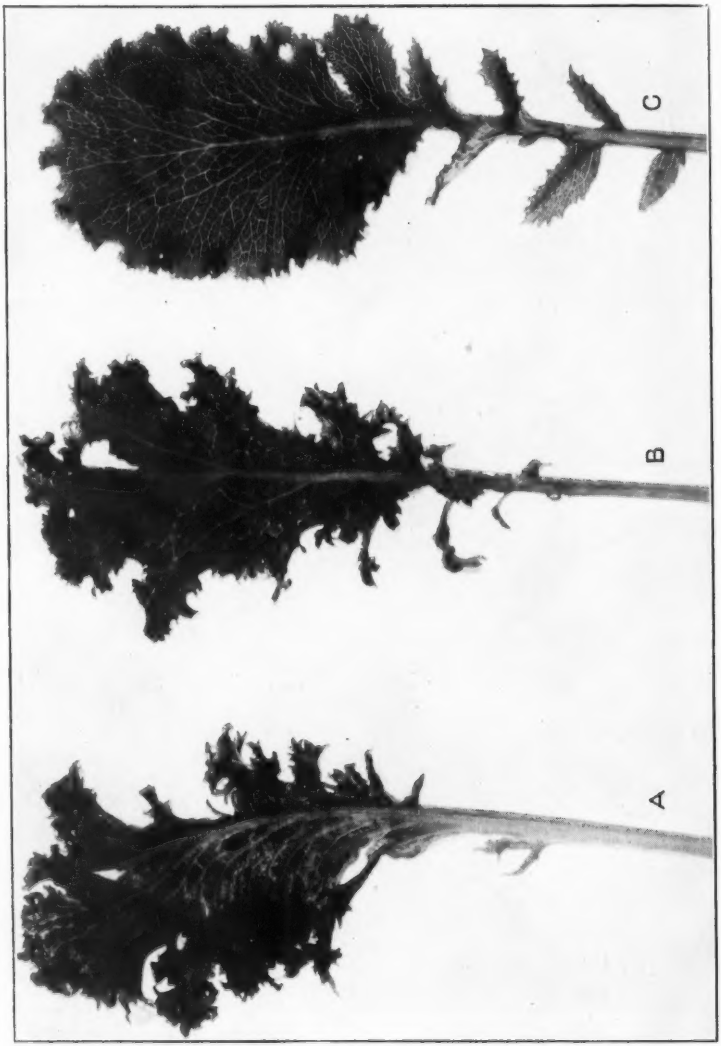
C
B
A

PLATE 24

Leaves from plants shown in Plate 23, A, B.
A, B.—Mosaic leaves showing mottling and ruffling.
C.—Healthy leaf.